Abstracts of

Royan International Twin Congress

10th Congress on Reproductive Biomedicine
5th Congress on Stem Cell Biology & Technology

23-25 September 2009

ROYAN INSTITUTE

Tehran, Islamic Republic of Iran

Yakhteh Medical Journal (The Cell)

Guide for Authors

Aims and Scope: The "***Yakhteh Medical Journal (The Cell)***" is a publication of Cellular Sciences Research Centre, the 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.

This journal accepts (Original articles, Review articles, Case reports, Short communications, Editorial, Images in Biology papers and Letter to editors) in the field of cellular and molecular.

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.

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

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To minimize such errors author should verify references against the original documents.

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* Name(s) of author(s).Manuscript title. Journal title (abbr).publication date (year); Volume &Issue: Page number.
* Manicardi GC, Bianchi PG, Pantano S, Azzoni P, Bizzaro D, Bianchi U, et al. Presence of endogenous nicks in DNA of ejaculated human spermatozoa and its relationship to chromomycin A3 accessibility. Biol Reprod .1995; 52: 864-867.

Book:

* Name(s) of author(s).Book title. Edition. Publication place: publisher name; publication date (year); Page number.
* Edelman CL, Mandle CL. Health promotion throughout the life span. ST Louis: Mosby; 1998; 145-63.
* Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995; 465-78.
* Sigman M, Lipshultz LI, Howards SS. Evaluation of subfertile male. In infertility in the male. Lipshultz LI, Howards SS (eds).Philadelphia: Mosby Year Book; 1991; 179-210.

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Eftekhati Yazdi P, Comparison of Fragment Removal and Co-Culture with Vero Cell Monolayers on Development of Human Fragmented Embryos. Presented for the Ph.D., Tehran. Tarbiyat Modarres University. 2004.

Abstract book:

* Amini rad O. The Antioxidantal Effect of Pomegranate Juice on Sperm Parameters and Fertility Potential in Mice.Yakhteh.2008;10 Suppl 1:38.

Conferences:

* Harnden P,Joffe JK, Jones WG,editors.Germ cell tumors V.Proceedings of the 5th Germ cell tumors conference ;2001 Sep 13-15;Leeds,UK. New York:Springer;2002.

Internet References

Articles:

* Jahanshahi A, Mirnajafi-Zadeh J, Javan M, Mohammad-Zadeh M, Rohani M.Effect of low-frequency stimulation on adenosineA1 and A2A receptors gene expression in dentate gyrusof perforant path kindled rats. Yakhteh. 2008; 10(2): 87-92. Available from: http://www.yakhteh.org. (20 oct 2008).

Book:

* Anderson SC, Poulsen KB. Anderson's electronic atlas of hematology. [CD-ROM]. Philadelphia: Lippincott Williams & Wilkins; 2002.

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.

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Abbreviations and symbols: 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 do not need to be defined. Avoid using them in title and abstract.

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3. References mentioned as stated in the Instruction to Authors section.

4. Tables should be typed on separate pages.

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8. Letter of Undertaking signed by all authors.

9. Disclosure regarding source of funding and conflict of interest if any besides approval of the study from respective Ethics Committee/ Institution Review Board.

10. Covering Letter

11. Floppy disk (latest version of windows) for on-line edition

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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.

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Abstracts of Royan International Twin Congress 10th Congress on Reproductive Biomedicine 5th Congress on Stem Cell Biology & Technology

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Scientific Board

Collaborator

- Iranian Stem Cell Network
-Iranian Society for Reproductive Stem Cell
- Stem Cell Initiative
-Iran University of Medical Science
- Akbarabadi Hospital, Iran University of Medical Science
- Tehran University of Medical Science
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    - Digestive Disease Research Center of Shariati Hospital, Tehran University of Medical Science
    - Infertility Center of Shariati Hospital, Tehran University of Medical Science
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- Shiraz University of Medical Science
    - Zeynabiye Fertility Center, Shiraz University of Medical Science
    - GasrtoEntroHepatology Research Center, Shiraz University of Medical Science
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- Bqiatallah Hospital, Bqiatallah University of Medical Science
- Shaheed Rajaie Cardiovascular , Medical & Research Center
-`Fatemiyeh fertility center, Hamedan University of Medical Science
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-Sasan Hospital
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- Isfahan Fertility Center
- Mehr Infertility Institute of Rasht
- Omid Fertility Center
- Sarem Fertility Center
- Rasht Family IVF Center
- National Institute for Genetic Engineering and Biotechnology

Congress Chairman

Ashraf Moini

Dear Colleagues and Friends,

It is my privilege and honor to welcome you to Royan's International Twin Congress: the 10th Congress on Reproductive Biomedicine and the 5th Congress on Stem Cell Biology and Technology.

The Executive Committee has tried once more to organize an interesting scientific meeting thanks to the international contributions of prominent professors and keynote speakers who will be participating. High level oral and poster presentations, which have been selected from numerous international applicants, will make this event a good forum for the exchange of knowledge and in-depth discussions on the latest advancements and ongoing research in a background of international developments.

I also would like to extend my appreciation and thanks to all members of the Scientific Board and Executive Committee for their continuous efforts and indefatigable endeavor which will certainly make this scientific event a great success.

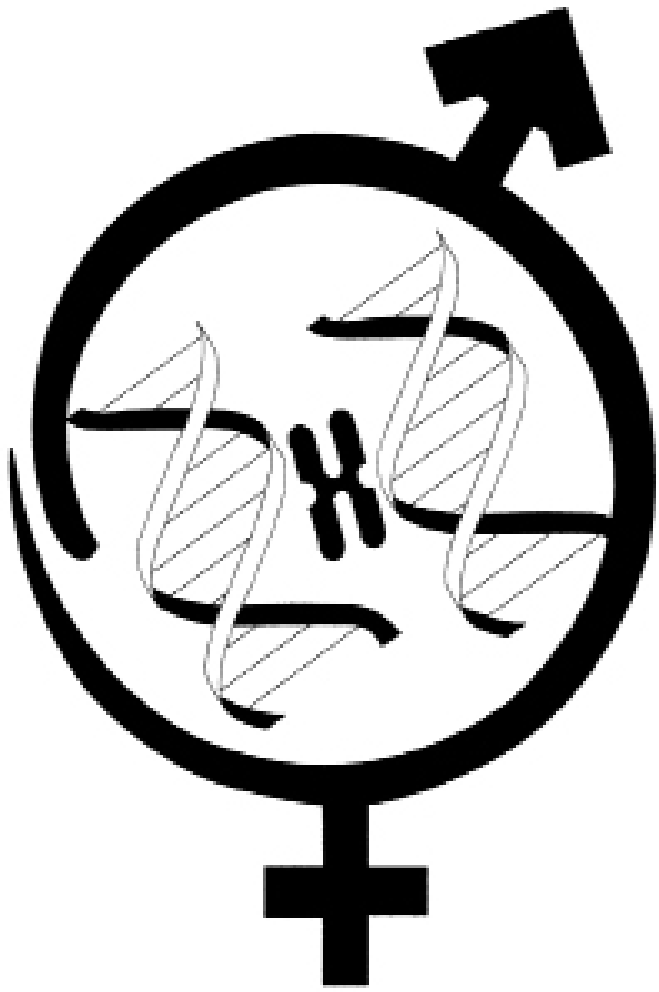
Last but not least, I wish for all to benefit from this opportunity and visit the ancient historical and cultural heritage of Iran during a pleasant and enjoyable stay in this unique Middle Eastern country.

Ashraf Moini M.D.
Congress Chairman

Abstracts of

10th Congress on Reproductive Biomedicine

23-25 September 2009



ROYAN INSTITUTE

Invited Speakers

Embryology

I-1: Cloning and Transgenic Animals Research in Iran

Nasr Esfahani MH

Department of Reproduction and Development, Royan Institute for Animal Biotechnology, ACECR, Isfahan, Iran

In 1980, the first transgenic mice called the "super mice" capable of producing a human drug, tPA (tissue plasminogen activator to treat blood clots), was produced by the pronuclear microinjection (PNM) technique. However, with the birth of 'Dolly' and 'Polly' in 1997, the methodology of livestock transgenesis was greatly revolutionized. Dolly, of course, is the famous 'cloned sheep' announced in early 1997, and 'Polly' is a transgenic sheep from the same research program. Since then, isolation of human pharmaceutical proteins from milk of genetically modified animals became a priority for all the countries. Accordingly the cloning animal using the delicate technology of somatic cell nuclear transfer (SCNT) has expanded from handful research laboratories in to over 200 laboratories across at least 40 nations.

This perspective describes a historical background of the current status of cloning and transgenesis technology in Iran regarding the worldwide's developments. We outline the experimental designs, the pitfalls and challenges encountered, and the eventual success in developing a practical approach. Finally, our suggestions of the promises of these two tightly joined technologies for advancing basic research science and pharmaceutical and therapeutic strategies will be discussed.

I-2: Oocyte-Somatic Cell Interactions and Implications for Fertility Regulation

Kenneth P McNatty

School of Biological Sciences, Victoria University of Wellington, New Zealand
Email: ken.mcnatty@vuw.ac.nz

In mammals, the initiation of ovarian follicular growth occurs as the oocyte enlarges and the immediately adjacent somatic (i.e. granulosa cells) develop a uniformly cuboidal appearance and proliferate. Whilst this transformation is accompanied by the expression of several hundred new genes not expressed in the primordial follicles, the key factors responsible for the initiation of growth are not well understood. Nevertheless, it has now been established that the oocyte plays an essential role in regulating follicular growth once growth has been initiated, at least until follicles develop a dependency on the pituitary hormones, LH and FSH for preovulatory maturation. During the period preceding gonadotrophin dependence, the oocyte is thought to regulate follicular growth through the secretions of growth factors such as bone morphogenetic protein (BMP)-15 and growth and differentiation factor (GDF)-9 and by interactions with the granulosa cells via an extensive network of gap-junctions. In this way, the oocyte is thought to regulate the proliferation and differentiation of its adjacent granulosa cells into two distinct phenotypes, namely, the cumulus cells (those immediately adjacent to the oocyte) and the mural granulosa cells (those more distantly located from the oocyte). Moreover, from studies in sheep, it appears that the localised concentrations of BMP-15 within the developing follicle influences the timing of formation of receptors for LH on mural granulosa cells and thus the number of follicles that may go on to ovulate. Currently, there is intense interest in determining the molecular forms and mechanisms of action of BMP-15 and GDF-9 and the potential applications of these growth factors for improving fertility in vivo and for use with *in vitro* technologies to improve IVF outcomes

I-3: Genetic Mutations in Sheep Influencing Ovarian Follicular Growth and Ovulation-Rate

Kenneth P McNatty

School of Biological Sciences, Victoria University of Wellington, New Zealand
Email: ken.mcnatty@vuw.ac.nz

Studies of the genetics of ovulation-rate have proven helpful for understanding the physiology of ovarian follicular function in mammals. Since 2000, studies of the inherited patterns of ovulation-rate in sheep have revealed at least 6 different point mutations in either the pro- or mature protein regions of bone morphogenetic protein (BMP)-15 and at least two different point mutations in the mature protein region of growth and differentiation factor (GDF)-9. Animals homozygous for any of the X-chromosome derived BMP-15 mutations (e.g. FecXB) and one of the GDF-9 mutations (FecGH) on sheep chromosome 5 are sterile with streak ovaries, whereas, those heterozygous have ovulation-rates approximately one higher than those of the wild-types. Animals heterozygous for both FecGH and FecXB have ovulation-rates that are additive for each mutation separately indicating that these growth factors can co-operate with one another. Within the sheep ovary, both BMP15 and GDF9 are expressed exclusively by oocytes. These findings together with those from other studies in cattle and humans show that the oocyte has a major role to play in regulating ovulation-rate in mammals. In addition to BMP15 and GDF9, a point mutation in a BMP type I receptor known as activin-like kinase (ALK)-6, on sheep chromosome 6, also has a profound influence on ovulation-rate. Animals hetero-

zygous and homozygous for the ALK-6 mutation have ovulation-rates that are 1.5- and 3.5-fold higher respectively, than their wild-types. As with BMP-15 and GDF-9, the ALK-6 gene is expressed in oocytes, but also in granulosa cells of developing ovarian follicles. Other mutations in sheep, known to effect ovarian follicular development and/or ovulation-rate, have been localised to chromosomes 5 (Thoka ewes), 11 (Lacaune) and X (Woodlands), but these genes remain to be identified. In the case of the Woodlands mutation (FecX2W), it seems that this also affects ovulation-rate at the level of the ovary as the patterns of expression of the BMP-15, ALK-6 and ALK-5 genes in oocytes or granulosa cells differ between those carrying the FecX2W mutation and the wild-type.
In summary, a remarkable range of naturally-occurring ovulation-rates varying between 0 and 15 have been identified in domesticated sheep. All the genes identified as responsible for this diversity are expressed in oocytes and/or adjacent follicular cells. An understanding of how these factors influence follicular development, ovulation rate and fecundity are currently the subject of intense research.

I-4: Ovarian Tissue Cryopresrrvation and Grafting an Ultrastructural Approach

Stefania A. Nottola

Department of Anatomy, Laboratory for Electron Microscopy "Pietro M. Motta", Faculty of Medicine, University of Rome "La Sapienza", Rome, Italy
Email: stefania.nottola@uniroma1.it

Cryostorage and grafting of frozen-thawed (F/T) isolated ovarian follicles, ovarian tissue fragments or whole ovaries are all possible therapeutic approaches now available to preserve fertility in young patients undergoing potentially gonadotoxic radio/chemotherapic treatments for cancer or other systemic pathologies. Our aim was to evaluate through a morphological approach the impact of enzymatic isolation, cryopreservation and grafting on ovarian tissue integrity and viability. Human ovarian tissue was analyzed after the following treatments: 1- Follicle isolation by collagenase or Liberase enzymatic digestion; 2 - Xenotransplantation and orthotopic autotransplantation of F/T ovarian cortical strips; 3 - Cryopreservation of intact ovaries with their vascular pedicles. Observations were carried out by transmission electron microscopy, vital fluorescent staining, immunohistochemistry and DNA strand breaks analysis by TUNEL. In treatment 1, a higher proportion of follicles were viable after Liberase isolation in respect to those isolated with collagenase, and most of Liberase-treated follicles were of good ultrastructural morphology. These data imply that the treatment with Liberase - a purified, endotoxin-free enzyme blend - is a promising alternative to impure collagenase preparation for the isolation of intact ovarian follicles for culture and grafting purposes. In treatment 2, cryopreservation and transplantation do not appear to greatly affect the morphology of human primordial/primary follicles, which even grow in the grafts; however, follicular density was reduced after transplantation and follicular development appeared initially impaired. Thus, further studies are needed to extend follicular life span and to improve follicular growth in the graft. In treatment 3, cryopreservation was not associated with any particular sign of apopotosis or ultrastructural alteration in all ovarian (follicular, vascular and stromal) compartments. This result suggests that whole-organ sampling and transplantation may be a viable option in the future, since vascular pedicle anastomosis allows immediate revascularization and greatly reduces ischemic injuries in the transplanted tissue.
Funds were provided by Italian Ministry of Education, University and Research (university grants) and by Italian Ministry of Foreign Affairs.

I-5: Ultrastructure of Human Preovulatory Oocyte in Assisted Reproduction Protocols

Stefania A. Nottola

Department of Anatomy, Laboratory for Electron Microscopy "Pietro M. Motta", Faculty of Medicine, University of Rome "La Sapienza", Rome, Italy
Email: stefania.nottola@uniroma1.it

In the field of assisted reproduction technology oocyte cryopreservation protocols have not been fully optimized yet and overall clinical success remains relatively suboptimal. Further experimental (morphological, biochemical, biomolecular) and clinical studies seem thus necessary, in order to better comprehend the effects on human oocytes of all factors associated with freezing and ultimately tailor the best protocol for human oocyte cryopreservation. Our aims were to evaluate the ultrastructure of human preovulatory oocytes frozen-thawed (F/T) using different protocols of cryopreservation and to compare the data obtained with the morphology usually shown by fresh (control) oocytes. In detail, our study investigated the ultrastructural features of oocytes subjected to various procedures of slow cooling and vitrification. Particular emphasis has been given on how the vitrification process impacts the ultrastructural morphology of the mature oocyte. In fact, vitrification currently offers interesting perspectives in the field of oocyte cryopreservation, being judged more tolerable than slow cooling by the oocyte. Despite this, very little is known about the fine morphology of vitrified-warmed oocytes, and the available studies are almost totally concerning non-human species. F/T oocytes were vitrified using two alternative devices (cryoloop and cryoleaf). By light microscopy both fresh and F/T oocytes were generally rounded, 90-100 microns in diameter, provided with an ooplasm showing a uniform texture and surrounded by a continuous zona pellucida. Sparse microvacuolization was only occasionally detected in both fresh and F/T oocytes, at the same ex-

tent. Transmission electron microscopy (TEM) evaluation confirmed the very sporadic incidence of ooplasmic vacuolization in fresh and F/T oocytes. By TEM, oocyte organelles mostly consisted of mitochondria-smooth endoplasmic reticulum (M-SER) aggregates, varying in shape, size and location. In particular, in more than a half of the F/T oocytes observed, M-SER aggregates appeared slender in shape and smaller in size than those observed in fresh oocytes; in spite of this, a proper mitochondrial fine structure was generally maintained in all these samples. In the remaining F/T oocytes, and in fresh oocytes as well, both small and large aggregates were found randomly distributed in the ooplasm. Finally, numerous F/T oocytes displayed a non-homogeneous distribution of microvilli and a reduced complement of cortical granules (CGs) – the latter particularly evident in those oocytes vitrified using the cryoloop device. In conclusion, a) the virtual absence of ooplasmic microvacuolization seems the most relevant marker of quality in vitrified-warmed oocytes, irrespective of the device utilized; b) CG pattern appears better preserved in cryoleaf-supported oocytes; c) the finding of underdeveloped M-SER aggregates and altered microvilli emphasize the need of further ultrastructural studies on human vitrified oocytes.

Funds were provided by Italian Ministry of Education, University and Research (university grants) and by the Italian National Health Institute.

Epidemiology and Ethics

I-6: An Iranian Perspective on the Conflict between Human Dignity and Surrogate Motherhood

Aramesh K

Medical Ethics and History of Medicine Research Center
Tehran University of Medical Sciences
Email: kiarasharamesh@sina.tums.ac.ir

Surrogacy motherhood, as a method of assisted reproduction, is being widely practiced in Iran. The acceptance and legitimacy of this practice, in the absence of pertinent rules and regulations, is according to the Jurisprudential Decrees (Fatwas) obtained from some of the Shiite religious authorities. According to these Fatwas, commercial surrogacy is being practiced in Iran. Commercial surrogacy, in contrary to the altruistic version, seems to be in conflict with the very concept of human dignity.

Human dignity, as a notion rooted in both secular and religious schools of thought, can be regarded as a common platform of ideas among the main existing schools of moral philosophy and ethics. This concept can be relied on, for reaching to common principles and norms among all these schools.

According to the Shiite theology, notions such as human dignity could have independent logical basis regardless of their roots in the Holy Scripture. Therefore, human dignity can be considered as a concept which is found by reason and emphasized by the scripture. However, it seems that in the current jurisprudential discourse among religious scholars, when the bioethical issues are being discussed, more attention and observation to this principle is needed.

In this article, after a brief situation analysis of the current practice of surrogacy in Iran, I will assess the jurisprudential and ethical views toward the altruistic and commercial surrogacy and will conclude that although the altruistic one is acceptable by both Shiite jurisprudence and medical ethics, the commercial surrogacy, because of its conflict with human dignity, arises some major ethical concerns and a revision of jurisprudential decrees in this regard is necessary.

Also, I will argue that surrogate motherhood can be discussed as an example showing the necessity of the observance of the concept of human dignity in the future of Islamic bioethical jurisprudence.

I-7: Ethical Issues in Gamete Donation

Eric Blyth

University of Huddersfield, Huddersfield, UK
Email: e.d.blyth@hud.ac.uk

Semen donation is one of the earliest – and technologically least sophisticated - forms of assisted reproduction, with evidence of physicians offering the service at least as far back as the mid 19th century CE. However, from the outset, the dubious acceptability, morality and legality of the practice ensured that it was practiced as a clandestine rather than simply a confidential medical service. Physicians ensured that donors (frequently their friends and professional colleagues) and recipients never knew of each other's identity, often advised recipients and their partner not to tell anyone – including their child(ren) – of their recourse to semen donation, and no provisions were made for donor-conceived people to learn anything about their donor. Secrecy, anonymity and donor recruitment practices promoted the use of semen from a single donor on multiple occasions, and it is known that some donors have helped to conceive many dozens of offspring. As demand for semen donation increased, physicians sought to recruit new donors, offering modest financial reward as an incentive. Subsequent technological developments enabled oocyte and embryo donation to become widely available, applying similar practice models to those already adopted for semen donation, although higher levels of financial compensation have tended to be offered to oocyte donors. While semen, oocyte and embryo donation were initially intended to assist heterosexual couples with a fertility difficulty to conceive a child, they have become a key means by which single women and lesbian couples (using donated semen) and post-menopausal women (using donated oocytes or embryos).

Although comparatively little empirical information concerning gamete donation is available, primarily because it was set up in a way that precludes the accumulation of an adequate evidence base, a number of ethical issues have emerged and which will be discussed in this presentation, including:
• Service providers' responsibilities to promote evidence based practice
• Service providers' responsibilities towards donors, recipients and donor-conceived individuals
• Welfare of donor-conceived individuals-including
• Separation of children from their genetic parent(s)
• Limits on the number of offspring conceived by the gametes of a single donor
• Information for donor-conceived individuals about their genetic and biographical history and about their genetic relatives
• Use of gamete and embryo donation by single women, individuals in same sex relationships and older, postmenopausal women.
• Donors' responsibilities and obligations
• Commercialisation of gamete procurement and financial rewards for donors
• Risks of donor exploitation

I-8: Welfare of the Child in Assisted Reproduction

Eric Blyth

University of Huddersfield, Huddersfield, UK
Email: e.d.blyth@hud.ac.uk

Over 3.5 million children are believed to have been born worldwide – and over 200,000 annually - as a result of assisted reproduction procedures. Such procedures account for an increasing proportion of all births; for example, among European countries where data are available, approximately 1.7% of all births each year result from assisted reproduction procedures.
It is commonly asserted that the welfare or interests of children born as a result of assisted reproduction procedures should be a high priority of service providers - a requirement that is enshrined in statute in some jurisdictions. Such requirements have tended to focus exclusively on the suitability of potential parents of children conceived through assisted reproduction procedures; this focus has highlighted the difficulties of operationalising both welfare principles and requirements.
However, the impact of specific assisted reproduction procedures on children has received less attention, and while it is frequently claimed that assisted reproduction procedures have become routine practices, the underpinning evidence base, especially regarding the long-term safety of assisted reproduction procedures, is less robust than for other similarly well-established procedures.
This presentation will highlight these issues by drawing on the specific examples of intracytoplasmic sperm injection (ICSI), multiple births, pre-implantation genetic diagnosis (PGD), selecting the characteristics of children, and use of previously cryopreserved embryos, oocytes, and semen.
The presentation will identify key challenges that need to be addressed to further promote the welfare of children conceived through assisted reproductive procedures:
• Accurate information and comprehensive national registration systems
• Further research to identify longer-term health, psychological and social implications of assisted reproductive procedures on children and their families
• Establishment of formal requirements to take account of the welfare of children conceived through assisted reproductive procedures.

I-9: The Role of Biomarkers in Reproductive Epidemiology

Kamali K

Email: kkamali71@yahoo.com

Biological markers are material measures obtained from the bodies or excretions of individuals potentially usable to detect: environmental exposures, effects of exposures.
The rationale for biological markers in epidemiologic research is strong in that markers have the potential for improving the accuracy of our "exposure variables", permitting the identification of preclinical disease and providing opportunities for prevention, allowing for more homogeneous and etiologically relevant classifications of disease, and enhancing our understanding of the biological processes leading to disease occurrence.
When we are dealing with biomarkers, they need validation using laboratory, epidemiologic, and clinical studies. The analytic validity, clinical validity and clinical utility of a biomarker are important for evaluating it.
The use of biomarkers in epidemiologic research raises a number of ethical, legal, and social issues.
Biomarkers in reproduction epidemiology are considered as markers of events before and around fertilization, biological markers in discovering or interpreting female reproductive disorders that might be owed to environmental causes, markers of the pre- and peri-implantation phases and markers of the postimplantation phase, experience with studies of chromosome anomaly in spontaneous abortion.

I-10: Posthumous Reproduction and Donation, Islamic and Iranian Perspective

Reza Omani Samani, Mohammad Reza Rezania Moalem

Department of Epidemiology and Reproductive Health, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: samani@royaninstitute.org

Posthumous assisted reproduction is the most challenging, difficult and sensitive issue to be discussed ethically and religiously. In this paper the acceptability of the posthumous reproduction in Islamic contexts is evaluated and major concerns like Consent and ownership of the gametes after death, Family and Marriage vision and Welfare of the child are discussed together with some international legislation. We can conclude that upon Islamic vision to assisted reproductive techniques as treatment of families and relieving the serious problem of childlessness, posthumous assisted reproduction is unacceptable for the remaining partner even with previously frozen gametes or embryos. Also, Islamic vision to marriage, consent and welfare of the child confirms the unacceptability. Posthumous gamete or embryo donation is different. The frozen embryos or gamtes after the person's death can save another infertile family with less problems. As the parent is dead, upon current law, after one year, there is no lineage related to the child who is born. So, problems like intimacy, inheritance, expenditure and guardianship will be solved. It seems before starting such a program, there is an extreme need for a complete research and obtaining decrees and pursue a complete guideline preventing any future problem.

Keywords: Posthumous, Reproduction, Islam, Donation

I-11: Donation, Surrogacy and Payment, Indian Experience

Roya Rozati

Medical Director, MHRT Hospital and Research Centre, Hyderabad, INDIA
Professor and Head, Department of Obstetrics & Gynecology, Owaisi Hospital and Research Centre, Hyderabad, INDIA.
Email: drroyarozati@gmail.com

Recent advances in reproductive technologies have transformed the way people look at having children. Childless couples need not, and do not perceive their life a cursed one, thanks to the increasing practice of donor insemination, egg donation, *in vitro* fertilization, embryo transfer, surrogacy and its acceptance. Surrogacy is now a viable clinical option for childless couples. The two practices Egg donation and Surrogacy or contract pregnancy as some prefer to call it, raise very different ethical issues along with payment for Surrogacy.
It has become evident that it is generally the socio-economically marginalized women who agree to act as surrogates due to the financial benefit it entails. This not only puts these already-vulnerable women in situations where their bodies may be exploited for the benefit of other people, but also jeopardizes their physical and mental health, thus making them 'objects of reproduction'. Donors should not be paid for their eggs, but rather they should be compensated for the burdens of egg retrieval. Making the distinction between compensation for burdens and payment for a product has the advantages of limiting payment, not distinguishing between donors on the basis of their traits, and ensuring that donors are paid regardless of the number or quality of eggs retrieved.
India is a hot destination for surrogacy because not only does it have a lot of successful IVF Surrogacy patients and good doctors there are a lot of women who are ready to be surrogates. The cost is the most beautiful part. Where the process takes about $60,000 in U.S, it is done is as much as $3000 in India. Who won't take this offer up? Who won't want to have a child in their arms after trying for years?

I-12: Legislation for Regulating ART Clinics in India

Sharma RS

Deputy Director General (Senior Grade) & Scientist-F, Div. of Reproductive Health and Nutrition, Indian Council of Medical Research, New Delhi - 110 029, India
Email: radheyss@gmail.com

The advent of any new scientific development that have wide applications and which impinge on human life raise several technical and moral dilemmas and poses many ethical and technical challenges. The assisted reproductive technology (ART) is no exception. ART also raises questions from society on their ethics and safety; in some instance moral issues also are raised. An additional factor arises when technology becomes privatized, with a possible loss of Govt. control. Unlike most other medical techniques, success rate using ART is poor. However, the desire of those whose marriages have remained barren, to have children, specially in our country, is so great that many infertility clinics with little expertise or reliability have come up all over the country. The services offered by some of these clinics are questionable. There is no formal training in this discipline in the country. The mushrooming of infertility clinics in India has been, therefore, a matter of great concern. This in turn creates pressure on politicians and legislators to scrutinize understand and control these practices. The reasons for this state of affair is the lack of National Guidelines and an Accreditation, Supervision and Regulatory body under which all infertility clinics offering ART would be placed. The Indian Council of Medical Research (ICMR) had developed "National Guidelines for Accreditation, Supervision and Regulation of ART Clinics in India" in consultation with the National Academy of Medical Sciences (NAMS) (India). The draft guidelines were widely publicized discussed and debated by expert groups of the ICMR and then by practitioners of ART and the public throughout the Country. The National Human Rights Commission (NHRC) and National Commission for Women (NCW) have also approved the guidelines. The

final guidelines have also been accepted by the Ministry of Health and Family Welfare, Govt. of India. To implement these guidelines in the country the guidelines have been translated into draft Assisted Reproductive Technologies (Regulation) Bill & Rules -2008 which was also subjected to public debate. Based on the comments & suggestions of various stake holders, both nationally and internationally and after consultation & approval of the experts the draft ART (Regulation) Bill & Rules has been revised and after the approval of the respective Ministries, Govt. of India it will be placed in the parliament of Republic of India for approval.
Artificial insemination by sperm (AID) has long been used to achieve successful pregnancies when the male partner is unable to father a child, or when there is a danger of transmitting a hereditary disorder to the offspring. After the development of IVF, the donation of eggs and embryos has also become a possibility. There are clear ethical parallels between sperm and egg donations. Like sperm, donor eggs can help infertile individuals achieve a pregnancy and have a child who is genetically related to one parent. In the case of Donor embryos, it is enabling a woman to go through pregnancy and childbirth. Even in surrogacy the child genetically belongs to either both the parents or at least one parent. However for some couples sperm or eggs might be thought to raise problems in so far as the social father or mother may feel less attached to a child that is not genetically his/her. This problem could be compounded by the use of donor embryos in few cases. But such problems should largely be able to be overcome by adequate counseling prior to the use of donor gametes or embryos.
One of the central ethical questions relating to egg, sperm and embryo donations is whether a child conceived in this way should or should not be told to her or his origin and what would happen in each case. Traditionally, as also in past, it was the almost universal practice to keep the identity of both the sperm donor and the recipient confidential. This practice has been challenged on the grounds that the child has a "right to know the truth", or that it is too difficult to hide the truth. Several countries now allow children when they reach majority to access at least non-identifying information about their genetic parents. Surrogacy is another important issue as infertile couples from different parts of the world comes to India for hiring Surrogate mother. There are number of issues both nationally and internationally in engaging surrogate mother and needs proper attention. Appropriate measures have been recommended in the Councils guidelines for ART Clinics in India as well as in draft Assisted Reproductive Technologies (Regulation) Bill & Rules -2009 on various issues related to AID, ovum donation & surrogate mother and will be discussed during the presentation.

I-13: Surrogacy and its Legal Regulation in the World

Konstantin N. Svitnev
Email: svitnev@jurconsult.ru

The right to reproduction is one of the fundamental innate human rights.
Surrogacy is the only way to overcome both biological and the social infertility, the only chance to have a child of one's own for millions of people in the world. Unjust and illogical bans deny people this right and lead to the so-called reproductive tourism.
The legal status of surrogacy and approach to this issue vary from one country to another. In a number of countries, such as Germany, France, Italy, Switzerland and Austria, surrogacy is prohibited by law. Other countries, such as Great Britain, Canada and certain Australian states allow surrogacy programmes only on a non-commercial basis, only the expenses linked to the surrogate mother can be reimbursed. Still other countries, like Russia, Ukraine, Belarus, Georgia, Moldova and a number of US states allow surrogacy on a commercial basis. Yet, in other countries, like Belgium, the issue of surrogacy is not stipulated by law, and the woman who gives birth to the child automatically becomes its mother.
In some Islamic countries surrogacy is not prohibited by law, yet it is not admissible from the point of view of the religious authorities.

I-14: Legal and Ethical Aspects of Posthumous Reproduction

Konstantin N. Svitnev

Email: svitnev@jurconsult.ru

Since the beginning of the human era, history has registered birth of the so-called 'posthumous' children. Most jurisdictions recognized these children as legal for a certain period of time after their fathers' death.
The breakthrough of assisted reproductive technologies has led to the development of extraordinary possibilities. Now a child can be born many years following the death of one or both of its biological parents.
'Posthumous' programmes are implemented through the usage of the cryopreserved material, often accompanying surrogacy and donation programmes. That is the scenario according to which in 2005 the first Russian 'posthumous' reproductive programme was realized for the Zakharovs family, who used the cryopreserved sperm of an unmarried young man who had died nine years before and a donor's oocytes. The child was carried be a'gestational' surrogate mother.
The posthumous reproduction manifests itself as the realization of the human right to reproduction even though after the death and the child's right to be born. Posthumous sperm collection is carried out by certain countries and has no legal ground, presuming that any human being would like to become a parent.
Today none of the countries in the world have a dis-

tinct legal stipulation for the implementation of such programmes, nor have they established the principles of registration of 'posthumous' children and protection of their legal and property rights.

Female Infertility

I-15: The Efficacy of Adding LH Supplement (Low dose HCG) in Late Follicular Phase for Folliculogenesis in PCO Patients

Ashrafi M

1. Department of Endocrinology and Female Infertility, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Obstetrics and Gynecology, Iran University of Medical Sciences, Tehran, Iran
Email: ashrafim@royaninstitute.org

Women affected with polycystic ovarian syndrome (PCOS) are the most difficult patients for a successful treatment with controlled ovarian hyper stimulation (COH). Their multiple and small antral follicles may be either resistant or highly sensitive to gonadotropin stimulation. Numerous controversies exist concerning the best protocols for folliculogenesis in PCOS patients.
One new approach for ovarian stimulation, which has been reported in some previous studies, is adding low-dose human chorionic gonadotropin (hCG) in the late follicular phase. The role of LH in sensitizing antral follicles to FSH is unclear. LH is required for normal hormone production and normal oocyte and embryo development, but follicular responses to LH may depend upon the stage of development. Low-dose hCG has a longer half-life and lower cost compared to recombinant LH. In addition, full development of large follicles, adequate ovarian hormonal level, oocyte maturation, avoidance of premature LH surge, and increment of pregnancy have been demonstrated by adding of low-dose hCG in late folliculogenesis.
Recently, Filicori et al showed that the completion of folliculogenesis could be achieved with the administration of low-dose human chorionic gonadotropin (hCG) in controlled ovarian hyper stimulation after 7 days of FSH-only priming. They have also reported a similar pregnancy rate with the use of this stimulation protocol in *in vitro* fertilization (IVF) with intracytophasmic sperm injection (ICSI). Traditionally, the role of LH in the control of the menstrual cycle was believed to be limited to stimulating theca cell androgen production, triggering ovulation and support of the corpus luteum. However, the physiologic selection of the dominant follicle in spontaneous menstrual cycles is now believed to be the result from the expression of LH receptors in the more mature ovarian follicles (>10 mm in diameter).
Ashrafi et al in their recent RCT compared the efficacy of two regimens of low-dose hCG (100 IU/ day or 200 IU/ day for folliculogenesis of PCOS women. These authors could not find any significant differences about stimulation duration and mean number of mature oocytes. However, immature oocytes were significantly lower in patients who received 100 IU hCG (group B). Study groups (groups B, C) also had lower gonadotropin consumption than the control group. Fertilization, implantation, and pregnancy rates were similar amongst the groups.
In summary, it seems that this new ovarian stimulation protocol permits follicles and oocytes to fully develop; helps generate top-quality embryos, avoids premature ovulation, establishes clinical pregnancies, reduces administration of recombinant hFSH, minimizes costs, and does not increase the chances of OHSS.

Keywords: Low dose hCG, Folliculogenesis, PCOS Women

I-16: Screening for Occult Maternal Conditons in Infertility Patients

Sarah L. Berga, James Robert McCord Professor and Chairman

Department of Gynecology and Obstetrics, Emory University School of Medicine, 1639 Pierce Drive, 4208-WMB, Atlanta, Georgia, USA 30322
Email: sberga@emory.edu

Stress is a generic term for a state of psychosocial disharmony and connotes a panopoly of meanings. Physiologically, stress is understood as activation of processes in response to events and conditions. Acute stress is associated with brief behavioral challenges that elicit adaptive neuroendocrinologic responses termed "homeostatic" that promote short-term survival. Chronic stress is associated with prolonged behavioral challenges. The neuroendocrinologic adaptations associated with chronic stress and protracted behavioral challenge are termed "allostatic." Allostasis is the achievement of stability through change and entails the alteration of the feedback "set point" for a given neuroendocrine arc.
Reproductive "alignment" occurs when physiological responses elicited by the external milieu modulate reproductive processes. A variety of factors serve as determinants and mediators of reproductive alignment. Because factors interact, the independent impact of any one factor may be minimal and difficult to discern. Indeed, a constellation of factors determines whether reproduction is promoted or impaired. Reproductive processes subject to environmental modulation include ovulation, fertilization, embryogenesis, implantation, placentation, and parturition. Clinical manifestations of reproductive misalignment include infertility, recurrent miscarriage, intrauterine growth restriction, preterm labor, low birth weight, preeclampsia, and congenital birth defects. Mechanisms mediating these

outcomes include alteration of physiological patterns of the hypothalamic-pituitary-adrenal (HPA) and -thyroidal (HPT) axes and alteration of genetic patterns, such as methylation of DNA. Physiological processes are generally viewed as plastic and reversible. While epigenetic alterations may be to some extent reversible, they also may be generationally transmitted. The term "fetal origins of adult disease" connotes disease or disorders due to transmissible epigenetic imprinting and underscored the notion that health promotion and predictive health begins before birth.

Not all maternal or paternal conditions that can compromise fetal well-being are readily recognized and include endocrine conditions such as subclinical hypothyroidism, impaired glucose tolerance, and diabetes, autoimmunity, infections, and genetic status. Stress is often an unappreciated cause of reproductive compromise and is difficult to recognize. While stress is generally categorized as metabolic or psychogenic, strictly speaking, these are not separable entities. Behaviors that activate stress signaling pathways activate pathways subserving metabolic and psychogenic outputs. Chronic activation of the HPA and/or suppression of the HPT can impede hypothalamic-gonadal function in both men and women. Individuals with functional hypothalamic hypogonadism typically display a combination of behaviors in response to ongoing psychogenic challenge that concomitantly induce mild energy imbalance and, in the context of chronic adrenal activation, increase the metabolic costs of common activities including recreational exercise.

Stress-induced anovulation (SIA), also referred to as functional hypothalamic amenorrhea (FHA), provides a classic and overt example of reproductive compromise and is characterized by the cessation of menses and infertility. SIA is accompanied by a constellation of neuroendocrine secretory aberrations and psychological correlates that include unrealistic expectations of self and others, perfectionism, and maladaptive attitudes about body image and food. However SIA represents only a small portion of the possible presentations for reduced gonadal function in women. Other, less overt, forms of reproductive compromise are far more prevalent and include polymenorrhea (menstrual interval < 24 days), oligomenorrhea (menstrual interval > 35 days), and luteal insufficiency with preserved menstrual interval. Occult reproductive compromise may only come to clinical attention if fertility is desired, especially in men.

While it is generally appreciated that maternal health impacts fetal health, the exact mechanisms mediating this link remain to be fully explicated. One must also recognize that factors with the potential to influence reproductive processes are not limited to those contributed by the parents. Medical interventions intended to facilitate reproduction also have the potential to activate these mechanisms. Thus, while assisted reproductive technologies allow us to bypass many of the mechanisms that mediate reproductive compromise, they also alter the developmental milieu during fertilization and early embryogenesis.

I-17: Postmenopausal Hormone Use and Brain Health

Sarah L. Berga

James Robert McCord Professor and Chairman, Department of Gynecology and Obstetrics, Emory University School of Medicine, 1639 Pierce Drive, 4208-WMB, Atlanta, Georgia, USA 30322

Email: sberga@emory.edu

Multiple mechanisms mediate the impact of hormones upon brain. Brain functions putatively impacted by hormones fall into categories such as motor, mood, and cognition. Hormones also have been implicated as hastening, protecting, or having nil effect upon molecular processes subserving neurodegeneration. Due to insufficient and contradictory findings, the debate over the importance of hormones for brain health continues unabated and clinicians and patients alike are left with few solid guidelines. Indeed, awareness that the brain is an important target tissue of steroids is still emerging. To complicate matters, drugs which modify or alter hormone levels have been added to the clinical armamentarium, but rarely has the impact of these hormonal mediators upon the brain been studied at molecular and clinical levels. Hence, drugs which modify or interdict hormone action are now in relatively widespread use while belatedly we are trying to understand the neurological, psychological, behavioral, and psychiatric implications.

Recently, we have focused on the impact of hormones upon brain neurotransmission using state-of-the-art neuroimaging approaches. In parallel, we have explored the impact of SERM's and AI's upon cognitive measures in women being treated for breast cancer. Both approaches confirm that hormones play a significant role in modifying neural substrates subserving cognition and emotion. In particular, it appears that aromatase inhibitors impart a more deleterious impact upon cognition than does tamoxifen in women being treated for early stage breast cancer. Further, serotonergic neurotransmission, which subserves cognition, mood, metabolism, and vegetative activities such as sleep, is modulated by physiological concentrations of estradiol and progesterone in young postmenopausal women. When considered in the context of epidemiological studies and other information about the molecular, cellular, and physiological functions of hormones, one must conclude that future studies that intend to delineate the pros and cons of hormonal modifiers should include the brain as one of the important target tissues. In particular, comparative neuroimaging studies are suggested as a means of determining which estrogenic substances might best support the postmenopausal brain.

I-18: Are All Estrogens the Same

Sarah L. Berga

James Robert McCord Professor and Chairman, Depart-

ment of Gynecology and Obstetrics, Emory University School of Medicine, 1639 Pierce Drive, 4208-WMB, Atlanta, Georgia, USA 30322
Email: sberga@emory.edu

With the advent of increasing molecular characterization of individual differences, it has become ever more important to understand how we might wisely individualize our approach to hormone use. The need to individualize, in turn, has spurred an interest in determining and understanding more about how estrogens differ. From the clinical point of view, we know that not all women are the same and we know that not all estrogens are the same. But which of the differences between women or hormones are the ones that really matter when making clinical decisions about birth control or menopause management?
When transdermal estradiol formulations for menopause management became available in the early 1990s, the dogma that all estrogens were equivalent was called into question. It was recognized that the transdermal route of administration of estradiol had less hepatic impact than the oral route and that this might confer benefits, including a reduced risk of venous thromboembolism. This line of reasoning seemed to make sense until a recent report (Cole et al. Obstet Gynecol 2007;109:339-346) showed that the transdermal contraceptive patch leads to higher rates of venous thromboembolism than oral contraceptives. Taken together, these observations beg the question as to whether route of administration of estrogens does indeed matter.
To dissect these findings, we must consider the pharmacologic attributes of different estrogen preparations and the physiologic mechanisms that are influenced by type, route of administration, and dosage of estrogen formulations. The goal is to illustrate the concept that not estrogens are the same and to highlight our knowledge gaps. We will likely be afforded a more clinically relevant perspective about the clinical differences between estrogens that are more chemically similar as we learn more about how each differentially harnesses intracellular machinery, such as protein chaperones.

I-19: Endometrial Factors in Recurrent Misscarriage

Marefat Ghaffari Novin

Cellular and Molecular Biology Research Center; Infertility and Reproductive Health Research Center, Shahid Beheshti University of Medical Science, Tehran, Iran
Email: mghaffarin@yahoo.com

Recurrent miscarriage is often defined as three or more consecutive miscarriages. The incidence of recurrent miscarriage among couples trying to conceive is 1–3%. Recurrent pregnancy loss may be a consequence of an abnormal embryonic karyotype, or maternal factors affecting the endometrium resulting in defective implantation. The causes for repeated implantation failure may be because of reduced endometrial receptivity. Various uterine pathologies, such as thin endometrium, endometriosis, hydrosalpinges, suboptimal ovarian stimulation, altered expression of adhesive molecules and immunological factors, may decrease endometrial receptivity.
In order to study the endometrial factors responsible for recurrent pregnancy loss, endometrial biopsy samples should be precisely timed according to the LH surge, and the investigation should be carried out in a non-conception cycle, prior to the next pregnancy. The various methods of studying the endometrium including morphological studies, morphometry, immunohistochemistry, measurement of endometrial protein in plasma and uterine flushings, cytokine expression in endometrial cells, leukocyte populations in the endometrium will be discussed.

I-20: Polycystic Ovary Syndrome, Lifestyle and Late Complications

Karimzadeh MA

Faculty of Medicine, Yazd University of Medical Sciences, Yazd, Iran
Email: makarimzadeh@yahoo.com

Objective: In this review article the evidence based data about the late complications of PCOS will be discussed. Lifestyle and exercise and management are the important points in control and prevention of the sequels.
Materials and Methods: Medline and Cochrane review.
Results: Polycystic ovary syndrome is associated with a variety of clinical disturbances including infertility, menstrual dysfunction, hyperandrogenism , elevated risk of pregnancy complications, and metabolic abnormalities such as increase in the prevalence of obesity, insulin resistance, and compensatory hyperinsulinemia, endometrial cancer and cardiovascular diseases. The problem of overweight and obesity is extremely prevalent in the PCOS population, and there is considerable evidence that obesity plays a negative role in the pathophysiology of PCOS. Lifestyle and genetics are the common predisposing factors in PCOS. The majority of studies support the positive effect of weight loss on improving risk factors for CVD and T2DM, reproductive endocrine parameter and outcomes in PCOS. In this lecture the role of exercise and diet in treating the syndrome, which though consistently recommended as the frontline therapy will be discussed. Lifestyle modification programs with an emphasis on behavioral management and dietary and exercise interventions have been successful in reducing the risk of diabetes and the metabolic syndrome in the general population and improving reproductive and metabolic features in PCOS. There is limited evidence for specific dietary and exercise approaches and guidelines for use in PCOS.
These strategies can be implemented into longer-term weight maintenance regimens through use of lifestyle modification techniques that consist of a multifaceted

approach of dietary, exercise, and behavioral therapies that aim to educate the individual with principles and techniques to achieve dietary and exercise. Alternatives to lifestyle management are anti-obesity pharmacology drugs such as metformin and bariatric surgery that they have limited and short time benefits for weight loss and lifestyle modification.

Conclusion: the most important aim of PCOS management is control and prevention of late onset complications and lifestyle modification and secrecies have proved to be critical in the management of PCOS.

I-21: Hysteroscopic Endometrial Embryo Delivery (SEED/HEED): Improving IVF Success Rate

Michael Kamrava[1]*, Asha Bhargava[2], Jerry Hall[3]

1. West Coast IVF Clinic, Beverly Hills, California 90212, USA
2. UCLA Geffen School of Medicine , Los Angeles, CA 90025, USA
3. LA Center for Embryo Implantation, Beverly Hills, CA 90212, USA
Email: drk@wcivf.com

Since the inception of *in vitro* fertilization (IVF), the procedure has seen many advances that have significantly improved pregnancy rates as well as a reduction in complication rates.

The benefits of blastocyst stage transfer have been established for the routine blind transfer technique of catheter introduction into the uterine cavity when a patient produces a large number of oocytes or has proven capacity to produce blastocysts. However, recent recommendations in using lower medication dosages for the controlled ovarian hyperstimulation, and in patients with lower response or advanced age, the number of developing embryos are limited and cleavage stage embryo transfers may be more advantageous. Also, since a significant number of embryos develop poorly beyond day three, early transfer is clinically prudent.

32 consecutive patients with Infertility of various origins underwent Hysteroscopic Endometrial Embryo Delivery (HEED) on day two or three or day five after fertilization. Controlled ovarian hyperstimulation was done using standard protocols. Transvaginal oocyte retrieval was performed under local anesthesia with mild sedation. All women received some type of luteal support, be it progesterone or hCG. Oocytes were fertilized and cultured in early cleavage medium (Irvine Scientific) at 37 degrees C and 5% CO_2 in air. Embryos were transferred at 48-120 hours post fertilization.

The percent of total ongoing live births per transfer is not different between transfer on day two and three, and percent of ongoing pregnancies occurring with transfers on day five is higher than transfers on day three or two. (Chi square value for day two vs. five = 1.36, $0.5<p<0.1$; chi square value for day three vs. five = 1.34, $0.5<p<0.1$).

Of pregnancies that occurred with transfer on day three, fifty percent resulted in miscarriage or biochemical pregnancy, while only sixteen percent had this result when transfer occurred on day two. Only one multiple pregnancy occurred with day of transfer two and five, while none of the transfers on day three produced a multiple pregnancy. Finally, no ectopic pregnancies were observed in either group.

These results using Hysteroscopic Endometrial Embry Delivery (HEED) show for the first time that the technique is very effective with both cleavage stage embryos and blastocysts, including day two embryos. These results are even more remarkable due to our clinical bias to replace embryos on day 2 when presented with poor quality or low numbers of embryos. Our findings could offer new hope of success for a large number of poor prognosis IVF patients.

I-22: Outcome of Different Preventive Strategies of Ovarian Hyperstimulation Syndrome in ART Cycles

Madani T

Department of Endocrinology and Female Infertility, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: tmadani@royaninstitute.org

Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic and life threatening syndrome with abdominal pain, nausea, vomiting, bloating, ascitis and sometimes with end organ damage resulting from excessive ovarian stimulation. The reported incidence of OHSS varies from 1% to 10% of *in vitro* fertilization cycles. Although ovarian hyperstimulation syndrome has been known for many years, it has been treated empirically and the underlying causes have been poorly understood. In order to reduce the risk of OHSS, several approaches have been proposed. One common technique is to coast the patient. In coasting, gonadotropin stimulation is withheld, and E2 levels are permitted to decrease before hCG is administered. Another technique, is elective cryopreservation of all embryos, plans to reduce the risk of OHSS by subsequent transfer of embryos in non-stimulated cycles. The present study compares the outcome of preventive strategies of ovarian hyperstimulation syndrome in ART cycles.

I-23: Ultrasound Assessment in Early Pregnancy Loss

Moini M

Department of Endocrinology and Femail Infertility, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

I-24: Thrombophilia and Recurrent Pregnancy loss

Moini A

1. Department of Endocrinology and Female Infertility, Royan Institute for Reproductive Biomedicine, ACECR,

Tehran, Iran
2. Department of Obstetrics and Gynecology, Faculty of Medicine, Tehran University of Medical Science, Tehran, Iran
Email: a_moini@royaninstitute.org

The role of inherited thrombophilias in RPL has generated a great deal of interest. This heterogeneous group of disorders results in increased venous or arterial thrombosis. Their associations with pregnancy loss rest on both proved and hypothetical alterations in placental growth and development, particularly placental vascular development. Abnormal placental vascularization and inappropriate placental thrombosis would link these thrombophilic states to pregnancy loss. Although some thrombophilic states may be acquired, most are heritable. Those heritable thrombophilias most often linked with reference to RPL include hyperhomocyteinemia,. Activated protein C resistance associated with mutations in factor V, deficiencies in proteins C and S, mutations in the prothrombin gene promoter, mutations in prothrombin, and mutations in antithrombin III.
Inherited thrombophilic mutations have been estimated to be causative in 50% of VTE during pregnancy. Approximately 40% of episodes of venous or arterial thromboembolic phenomena occur in patients carrying a heritable mutation. Associations between thrombophilias and adverse fetal outcomes cover a range of early gestational and obstetric disorders. These disorders include isolated and recurrent, early and late spontaneous pregnancy losses, intrauterine growth restriction (IUGR), intrauterine fetal demise (IUFD). Placental abruption, and pregnancy- induced hypertension (PIH). This discussion will focus on pathophysiologic mechanisms, diagnostic tesing, and treatment strategies for patients with RPL who may have an inherited or acquired predisposition to thrombosis (excluding the antiphospholipid syndrome).
The basis for the association between adverse fetal outcomes and heritable thrombophilias has focused on the mechanisms of impaired placental development and function secondary to venous or arterial thrombosis at the maternal-fetal interface.

I-25: Management of Endometriosis in Infertile Women

Parsanezhad ME

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

I-26: The Role of Endoscope in PCOS

Salehpour S

Faculty of Medicine, Shahid Beheshti University of Medical Science, Tehran, Iran
Email: saghar_salehpour@yahoo.com

PCOS is the commonest endocrinopathy in women and the commonest cause of unovutation in infertile couples.
The role of endoscopy in the diagnosis and treatment of PCOS is well know. With laparoscopy we can see PCOS ovaries, differentiate other causes of infertility such as tubal problems or pelvic adhesion.
With hysteroscopy we can see abnormal endometrial growth or polyps and we can do endometrial biopsy.
Beside diagnostic aid, endoscopy can be used in the treatment of PCOS.
In these cases we can do ovarian drilling with diathermy, Laser, crayon or hot water. And with this technic we induce ovutation, regulate the menstrual periods and increase the pregnancy rate.

I-27: Laboratory and Embryological Aspects in IVM Cycles

Weon-Young Son

McGill Reproductive Centre, Department of Obstetrics and Gynecology, Royal Victoria Hospital, McGill University, Montreal, Canada.
Email: weon-young.son@muhc.mcgill.ca

Despite a growing collection of translation research and the development of new clinical protocols, laboratory and clinical outcomes in IVM remain suboptimal when compared to the standard IVF. One of the most popular approaches in improving IVM success is to supplement follicular development with gonadotropin stimulation (FSH and/or hCG) prior to oocyte collection. In this presentation, recent advances in the laboratory and embryological aspects of IVM cycles are described including oocyte identification and characterization, *in vitro* maturation, insemination, timing of embryo transfer and cryopreservation.
In IVM cycles primed with hCG, oocyte identification is easier than in non- or FSH-primed IVM cycles due to the presence of an expanding cumulus mass around the oocytes. The immature oocytes with dispersed cumulus cells (CC) at collection in hCG-primed IVM cycles have high *in vitro* maturation and embryo developmental potential. Moreover, a few in vivo matured oocytes with dispersed CC can be obtained, and these have produced good quality embryos. Extending the period of hCG priming time before oocyte retrieval was one of the methods utilized to promote oocyte maturation in vivo and *in vitro*. The hCG could be given to patients when a dominant follicle (DF) reaches 10-12 mm to avoid detrimental effects on the sibling immature oocytes. ICSI has been suggested as the procedure of choice for inseminating mature oocytes and should be performed at least 1 h after the 1st polar body extrusion. Embryo transfer time depends on the quantity and the quality of the embryos produced after IVM. Vitrification is a more efficient method to freeze the embryos produced from IVM rather than slow freezing. In order to improve the IVM programs, it is essential to define not only the clinical aspects but also the laboratory and embryological aspects.

I-28: HCG Priming for IVM

Weon-Young Son

McGill Reproductive Centre, Department of Obstetrics and Gynecology, Royal Victoria Hospital, McGill University, Montreal, Canada.
Email: weon-young.son@muhc.mcgill.ca

A major side-effect of controlled ovarian hyperstimulation (COH) in patients with polycystic ovary or polycystic ovarian syndrome (PCOS) is the risk of ovarian hyperstimulation syndrome (OHSS). In-vitro maturation (IVM) of immature oocytes represents a potential alternative for the fertility treatment of these patients. Recently, applications of FSH (hMG) or hCG priming have been used before immature oocyte retrieval to improve the success rate of IVM procedures.
Although it is still controversial, hCG priming before oocyte retrieval seems beneficial in terms of easier oocyte retrieval, easier oocyte identification under stereomicroscope, maturation competence, and may increase the harvest of in vivo mature oocytes. Recently, new approaches attempted to improve the IVM program such as extending hCG stimulation or optimal hCG timing before immature oocyte retrieval. Therefore, as a first option, hCG-priming IVM treatment can be offered to women with PCO(S) instead of conventional IVF treatment with ovarian stimulation. In this session, I would like to introduce you to the recent progress of IVM cycles after hCG priming.

I-29: Implantation Failure Management

Taheripanah R

Obstetrics and Gynecology Department, Infertility and Reproductive Health Center, Shahid Beheshti Universty of Medical Sciences, Tehran, Iran
Email: taheripanahf@yahoo.com

Introduction: The purpose of this review is to evaluate the management of implantation failure following ART cycles. The main limitation of the successful IVF-ET cycle is the low implantation rate of the *in vitro* embryos that reduce success rate from the more than 90% embryo transfer to less than 30% pregnancy per started cycles. It is suggested that unknown processes occur in the black box of the uterus and for this reason the most investigation in the art field are done in this part of the treatments. Embryo , endometrial receptivity and systemic condition are the most important factors in implantation. Nowadays many clinical trials have been done to improve the success rate and overcome of the implantation failure.
Materials and Methods: Medline and Cochrane research.
Results: Preimplantation genetic diagnosis is one of ways that it is very useful for the patients specially older women. Aneuploidiy of the gametes and embryos is very common and it related to the maternal age. Assisted zona hatching may be overcome zona hardening in older women. Uterine cavity abnormalities and endometrial polyp disturbe the implantation. So ,hysteroscopic evaluation of the endometrial cavity even without any pathological finding increases the implantation rate by stimulation of the endometrial gland secretion and lymphokines. In this process endometrial injury has positive effect on ART outcomes. Thrombophilia is associated with lower success and implantation rate. Although anti-thrombotic treatment such as low dose aspirin, heparin suppress the immunological activities but no significant benefits is shown in some of the clinical trials and it is controversial.
Conclusion: PGD, correction of anatomical defect and increasing endometrial receptivity are the most important factors that can overcome on implantation failure and increase the success rate in ART cycles.

Keywords: Implantation Failure, Embryo, Endometrial Recpetivity

I-30: Endometriosis and ART

Taherinejad E. Sh

1. Department of Endocrinology and Female Infertility, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Faculty of Medicine, Tehran University of Medical Science, Tehran, Iran
Email: Tehraninejad@royaninstitute.org

Endometriosis is defined as the presence of endometrial glandular and stromal tissue outside the uterus that induces a chronic inflammatory reaction. It may be present in up to 22% of asymptomatic women and 30% of women with unexplained subfertility.
At present it is estimated that 10%–25% of all patients undergoing IVF are diagnosed with endometriosis, and 17%–44% of those also have ovarian endometriomas. *in vitro* fertilization has become the mainstay of treatment for endometriosis-related subfertility. Although there is some evidence to suggest that medical treatment with GnRH agonist can lead to a reduction in the size of the endometrioma by up to 51%, surgical removal of endometriomas remains the most effective approach for patients presenting with subfertility.
Despite the lack of a firmly established causal relation between endometriosis and infertility, it is clear that treatment of endometriosis can improve fertility in some cases. Expectant management may be a reasonable approach in younger patients with early stage disease and a shorter duration of infertility. Current medical therapy is not efficacious, and its use should be discouraged as it may only serve to postpone conception. Laparoscopic surgery appears to be superior to expectant management or medical therapy in minimal–mild endometriosis and may also be of benefit for patients with advanced endometriosis. COH/IUI is a good option in mild and surgically corrected disease. In pa-

tients with earlystage endometriosis, IVF outcomes are similar to those with unexplained or tubal factor infertility, and Gn-RHa treatment combined with IVF may be useful for more advanced disease.
In conclusion, the standard management of endometriosis in subfertile women before IVF remains controversial owing to the insufficient evidence to suggest superiority of one treatment strategy over another.
All the therapeutic options, including conservative, medical, or surgical treatment, as well as the advantages and disadvantages should be fully discussed with the patient. Any decision for surgery should be carefully considered and balanced against the risks, especially in women with previous adnexal surgery or women with suboptimal ovarian reserve. If the woman opts for surgical treatment, she should be appropriately counseled about the potential risks of reduced ovarian function after surgery, including the remote possibility of oophorectomy.

Keywords: Endometriosis, Infertility, Treatment, Pregnancy Outcome

I-31: Efficacy of Laparoscopic Treatment on 401 Patients with Infertility

YAO Shu zhong* , L IU Dong qing , WA N G Ning ning, HUA N G Jian zhao

Department of Obstetrics and Gynecology, First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China
Email: yszlfy@163.com

Objective: To assess the efficacy of laparoscopic treatment on women with infertility. Methods: Retrospective analysis of 401 women with infertility underwent laparoscopic operation according to the laparoscopic findings.
Results: The cumulative pregnancy rates were 50.0 % in the endometriosis group , 32.9 % in the tube obstruction group , 52.8 % in the polycystic ovarian syndrome (PCOS) group , 36.0 % in the uterine myoma group , and 42.0 % in the pelvic adhesion group respectively. 9310 %of women became pregnant within 18 months after operation.
Conclusion: Laparoscopy is a simple , timely and effective method for the treatment of female infertility If the patient remains infertile in 12 ~ 18 months after operation, it is suggested that the patient should be treated with other assisted reproductive techniques.

I-32: Factors Influence the Fertility Ability after Myometomy

Yao shu zhong

Department of Obstetrics and Gynecology, First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China
Email: yszlfy@163.com

Objectives: Uterine fibroids are one of the most common seen benign tumors of the uterus. The appearance of uterine fibroids has been linked to infertility in different degrees according to different locations, numbers, sizes and types. Considering surgery is the main treatment of uterine fibroids, the complications of surgery should be take into account. Postoperative adhesion formation which may influence postoperative fertility. There is still no sufficient evident about myomectomy's positive role in treating myomas. we analyze the different types, locations, numbers, sizes and many other factors related to myomectomy of fibroids' different impact on postoperative fertility of patients with infertile complaints before surgery, to find out myomectomy's role in treating infertility.
Materials and Methods: 78 patients enrolled in the study , including 15 among them who were diagnosed as infertile Patients were follow-up for 2 years postoperatively. Datas of almost every aspect associated with uterine fibroids and myomectomy were collected , and analyzed
Results: With the use of multivariable logistic regression analyze , age has a P valueof 0.02, with the OR value of 0.7 and the 95%CI (0.6, 1.0). This is the only independent factor which may influence the postoperative pregnancy rate, in a negative way. Other factors such as the presentation of infertility history, the location, size, number, entering the uterine cavity or not, types of the myoma and the surgery all have a P value of above 0.005 and the difference they have upon the postoperative pregnancy rate are not sinigficant.
Conclusions: To those who have uterine myomas, myomectomy may be an option for treatment, because postoperative pregnancy rate seems to increase significantly. Infertility may take place after the surgery for postoperative pelvic adhesion or potential factors that might cause problems on getting pregnant. For patients of whom uterine myomas seem to be the only problem, if have complaint of spontaneous abortion, problem may be solved after myomectomy. Age is the only independent factors that could influence fertility after surgery. The older the patient is , the harder can she get pregnant postoperatively. So early surgical treatment is recommended once myomas are found in the women in reproductive age. Myomas located in the anterior or posterior part of the uterine body have the similar impact on the postoperative pregnancy rate. Location, size, number, entering the uterine cavity or not, types of the myoma and the surgery do not affect the postoperative pregnancy rate.

Genetics

I-33: Chromosome Mosaicism throughout Preimplantation Human Embryo Development and its Clinical Significance

Asangla Ao

Department of Obstetrics and Gynecology, Royal Victoria Hospital, McGill University, Montreal, Quebec, Canada
Email: Asangla.ao@muhc.mcgill.ca

I-34: Chromosome Segregation in Embryos Obtained from Male Translocation Carriers Following Preimplantation Genetic Diagnosis

Asangla Ao

Department of Obstetrics and Gynecology, Royal Victoria Hospital, McGill University, Montreal, Quebec, Canada
Email: Asangla.ao@muhc.mcgill.ca

I-35: Cell-Mediated Transgenesis of Cattle for Biopharming and Dairy Products with Additional Health Benefits

Göetz Laible

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

Over millenia humans have shaped the genetic composition of today's livestock. Traditional breeding schemes and, more recently, marker assisted selection strategies have been successfully used for incremental genetic improvement of livestock. In principle, transgenic technology, which creates the potential to enhance existing characteristics at unprecedented magnitude and speed, can be seen as the logical progression from these more traditional efforts to modify livestock genomes. Unlike traditional breeding and selection, the technology is not restricted by the species barrier and can utilise the gene pool of other species to introduce entirely novel and unique characteristics. The recent development of cell-mediated transgenesis for livestock based on somatic cell nuclear transfer allows for the introduction of a wide repertoire of genetic modification. This includes not only additive strategies to introduce a new gene function (gain of function) but also the deletion of gene functions (knockout, loss of function), replacing a gene function with a different one (knockin, exchange of function) or the transfer of genes in a spatial-temporal manner (conditional knockout). The use of the mammary gland's high protein production capacity in dairy animals for the production of biopharmaceutical proteins in the milk has so far been the main driver for this technology platform due to strong economic incentive, ethical justification, greatest public acceptance and relative simplicity. The first biomedical product arising from a transgenic goat has recently been approved bringing transgenic technology into commercial reality. The genetic engineering of livestock provides also exciting opportunities to incorporate additional health benefits into important foods, such as milk, thereby creating entirely novel foods with unique properties not achievable by conventional means. Such food applications are however much more complex than biopharming and while presently in the research and development stage, their eventual introduction into the food chain remains some distance in the future.

I-36: Predictable and Stable Transgene Expression Using Recombinase Mediated Cassette Exchange and Episomal Vector Systems

Göetz Laible

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

Current transgenic livestock technology commonly relies on the random insertion of a gene construct into the genome. In this case, the activity of the transgene can be strongly influenced by the specific chromosomal context of the integration site, a phenomenon commonly referred to as position effect. As a consequence, the level of expression of a randomly inserted transgene can vary greatly or even result in adverse effects to the animals due to disruption of, or interference with, an endogenous gene. This unpredictability of transgene activity requires the generation and characterization of multiple transgenic cell and animal lines which is a major disadvantage for applications involving large animals due to the long gestation times and high costs involved. The randomness of the process can be avoided by the application of homologous recombination (HR) technology for the introduction of site-directed modifications. Due to the unavailability of livestock ES cells, HR approaches in livestock are presently restricted to the use of somatic cells which are hampered by very low efficiencies. The combination of the random chromosomal insertions of recognition sites for specific DNA recombinases (such as Cre or Flp) and the subsequent site-directed insertion of a transgene into this locus by a recombinase potentially offers much greater efficiencies. This recombinase mediated cassette exchange strategy provides a valuable system to efficiently produce transgenic livestock with predictable transgene expression levels. It also allows for the production of transgenic animals without antibiotic selection markers which are commonly used for the isolation of stably transfected cells but typically become part of the modified genome although they serve no useful function in the animal. An alternative approach is the use of episomal vectors which do not integrate into the genome but are maintained alongside the chromosomes as independent entities. They can thus ensure predictable expression and provide an elegant solution for potential problems associated with integration into the genome.
However, emerging episomal vector systems are commonly of viral origin and dependent on viral sequences and factors. This has major drawbacks because the viral elements may be recognised as invading DNA and

become permanently silenced or may even trigger the immune system, raising general biosafety concerns. The recent development of a novel self-replicating episomal vector system, based on the presence of a scaffold/matrix attachment region hold much promise as it can function independent of any viral factors. Moreover, the system has been engineered for the recombinase-mediated deletion of the bacterial vector backbone to limit the episomal vector that replicates in synchrony with the host cell chromosomes to its essential functional components. In the future, it will be important to demonstrate that these new concepts can live up to their promise and deliver greater predictability, efficiency and safety.

Oral Presentations

Andrology

O-1: Ethanol Induced Histological and Histochemical Changes in Testis, Sperm DNA Damage, Chromatin Integrity and Sperm Abnormality in the Experimental Model: Rat Model

Ahmadi A*, Sadrkhanlou R, Najafi G.H, Raberi A, Ghafari S, Kargar K, Ebrahimzade S

Embryology Department, Urmia university, Urmia, Iran
Email: dr.ahmadi2007@yahoo.com

Objective: Alcoholism in men causes increase in abnormal shaped sperms that can lead to impotency. Abnormal liver function and high estrogen levels may cause interference with sperm development which can severely suppress sperm. Moreover, alcohol abuse has been linked with damaged sperm and reduced sperm counts. . Like a toxin, alcohol may destroy the sperm-generating cells. In addition to this, alcohol abuse can also have adverse effects on the hormone levels in men. which may interfere with sperm development and hormone levels. Alcohol is also a toxin that can kill off the sperm-generating cells in the testicle. Worse still, chronic alcoholism can cause atrophy in the testicles, gynecomastia and the lack of sexual interest. Excessive alcohol consumption causes a disorder in the gonads resulting in changes in the structure of the testicles and decline in the T serum level. A drop in the T serum level can cause male infertility. In addition, alcohol can result in abnormalities in sperm size, shape and the sperm tail. Sperm motility can also be affected negatively. This change in sperm shape can seriously compromise the viability of the spermatozoa. Also, chronic alcoholism damages the sperm morphology that often has irreversible effects.
Materials and Methods: 42 adult male Wistar rats were randomly divided into 6 groups (n=7 each) with two groups serving as control, in the treatment groups were received 10% ethanol in distilled water for 35,70 and 120 days. All rats were sacrificed by CO2 inhalation and testis tissues were removed and prepared for Histological (H&E, Vaigrate Iodine and Toloedine blue(for mast cells) staining) and Histochemical (by cryosection and Lipase, Oil Red O staining methods) study. In addition spermatozoa were removed from cauda epididymis and analyzed for sperm motility, concentration in the cauda epididymis, viability and sperm chromatin quality and DNA integrity was assessed by Aniline blue and Acridine Orange staining following sperm sample preparation. Serum testosterone and estrogen level was determined by radioimmunoassay technique.
Results: This study confirmed that treated by Ethanol had significant decrease the testosterone level in plasma. . Ethanol had significant increase the DNA Damage and chromatin abnormality in the cauda epididymal spermatozoa as evidenced by Acridine Orange (AO) and Aniline blue staining respectively. Treatment of male rat with Ethanol caused significant decrease in sperm count, motility, and viability, while abnormal sperms increased as compared to control. Histological and histomorphometrical study confirmed that Ethanol had significant decrease the semniferous tubules diameter, number of germinal cells, and spermatogenesis in the semniferous tubules and increase the connective tissue and appearance of vacuolated edema in the interstitial connective tissue between the seminiferous tubules and with increased hypotrophic mass of leydig cells. Toloidine blue staining confirmed that Ethanol had significant increase the number of Mast cells in testis and epididymis tissues. Histochemical study by Oil Red O and Lipase methods confirmed that Ethanol had significant increase the fat droplets in the semniferous tubules and lipase reaction confirmed that the Ethanol had increase the lipase enzyme in the testis tissue.
Conclusion: Conclusion Yes, drinking alcohol can adversely affect on fertility and also cause damage to baby. The male reproductive system consists of the hypothalamus, the anterior pituitary gland, and the testes. Alcohol can interfere with the function of each of these components, thereby causing impotence, infertility, and reduced male secondary sexual characteristics. In the testes, alcohol can adversely affect the Leydig cells, which produce and secrete the hormone testosterone. Studies found that heavy alcohol consumption results in reduced testosterone levels in the blood. Alcohol also impairs the function of the testicular Sertoli cells that play an important role in sperm maturation. In the pituitary gland, alcohol can decrease the production, release, and/or activity of two hormones with critical reproductive functions, LH and FSH. Finally, alcohol can interfere with hormone production in the hypothalamus.

Keywords: Ethanol, DNA Damage, Histochemical, Histological, Acridine Orange, Testis

O-2: Effect of Sperm DNA Damage on Fertilization Rate Post ICSI

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

1. Anatomy Department, Isfahan Medical University, Isfahan, Iran
2. Department of Reproduction and Development, Royan Institute for Animal Biotechnology, ACECR, Isfahan, Iran
Email: razavi@med.mui.ac.ir

Objective: The first step for evaluation of male infertility is semen analysis but many studies suggest that other parameters such as sperm DNA integrity have more effects on ICSI outcomes. The aim of this study was to evaluate the correlation sperm DNA integrity using SCD and Comet assay with fertilization rate post ICSI.
Materials and Methods: DNA damage in sperm of 41 men undergoing ICSI treatment was measured by comet assay and SCD test. Around 16–18 h post ICSI, fertilization was assessed by presence of pronuclei and the percentage of fertilization was calculated by the ratio of fertilized

oocytes to the total number of survived injected metaphase II (MII) oocytes multiplied by 100 . Coefficients of correlation were calculated using SPSS 11.5 software.
Results: No significant correlation was obtained between fertilization rate and sperm DNA damage measured by SCD (p=0.091), and Comet assay (p=0.612) in ICSI patients .
Conclusion: Our results show that the sperm DNA damage doesn't directly effect on fertilization rate, since damaged DNA carried into the zygote by the fertilizing spermatozoon will be repaired by the oocyte. However, it can be effects on subsequent embryo development. It could be devised to identify and select spermatozoa with intact DNA or to remove spermatozoa with damaged DNA so as to improve ICSI outcomes.

Keywords: Comet Assay, SCD, Sperm DNA Fragmentation, Fertilization Rate

O-3: Effect of Smoking on Sperm DNA Integrity in Infertile Patients

Babazadeh Z[1]*, Razavi S[1], Nasr Esfahani M.H[2]

1. Anatomy Department, Isfahan Medical University, Isfahan, Iran
2. Department of Reproduction and Development, Royan Institute for Animal Biotechnology, ACECR, Isfahan, Iran
Email: razavi@med.mui.ac.ir

Objective: There is lots of oxygen reaction element in cigarette smoking that induce reactive oxygen stress (ROS) in the body ROS can make DNA damage in somatic and germ cells. The purpose of this study was to evaluate the effect of smoking on sperm DNA integrity in infertile patients by comet assay.
Materials and Methods: DNA damage in sperm of 72 men undergoing IVF and ICSI treatment was measured by comet assay and DNA damage was compared between smoker and nonsmoker groups by t-test using SPSS 11.5 software.
Results: The result show that significant different between sperm DNA integrity among smoker and non smoker groups (p=0.05).
Conclusion: it can be concluded that the cigarette prone sperm DNA damage.

Keywords: DNA Fragmentation, Sperm, Smoking, Comet Assay

O-4: PATZ1 Gene has a Critical Role in the Spermatogenesis and Testicular Tumors

Chieffi P[1]*, Chieffi G[1], Franco R[2], Paronetto M.P[3], Barbagallo F[3], Pero R[4], Chiariotti L[4], Fusco A[4], Fedele M[4], Salvatore G[5]

1. Dipartimento di Medicina Sperimentale, Naples, Italy
2. Istituto Nazionale dei Tumori "G. Pascale", Naples, Italy
3. Dipartimento di Sanità Pubblica, Rome, Italy
4. Dipartimento di Biologia e Patologia, Naples, Italy
5. Dipartimento di Medicina Clinica e Sperimentale, Naples, Italy
Email: paolo.chieffi@unina2.it

Objective: PATZ1 is a recently discovered zinc finger protein that, due to the presence of the POZ domain, acts as a transcriptional repressor affecting the basal activity of different promoters. To gain insights into its biological role, we generated mice lacking the PATZ1 gene. Male PATZ1-/- mice were unfertile, suggesting a crucial role of this gene in spermatogenesis. Consistently, most of adult testes from these mice showed only few spermatocytes, associated with increased apoptosis, and complete absence of spermatids and spermatozoa, with the subsequent loss of tubular structure.
Materials and Methods: The analysis of PATZ1 expression, by Northern blot, Western blot and immunohistochemistry, revealed its presence in Sertoli cells and, among the germ cells, exclusively in the spermatogonia.
Results: Since PATZ1 has been indicated as a potential tumour suppressor gene, we also looked at its expression in tumours deriving from testicular germ cells (TGCTs). Although expression of PATZ1 protein was increased in these tumours, it was delocalized in the cytoplasm, suggesting an impaired function.
Conclusion: These results indicate that PATZ1 plays a crucial role in normal male gametogenesis and that its up-regulation and mis-localization could be associated to the development of TGCTs.

Keywords: MAZR, ZSG, Spermatogenesis, Testicular Cancer, Tumour Suppressor

O-5: Evidence Based Medicine on the Pharmacologic Management of Premature Ejaculation

Mohammad RezaSafarinejad

Urology and Nephrology Research Center, Tehran, Iran
Email: safarinejad@unrc.ir

Objective: To evaluate the efficacy and safety of most popular selective serotonin reuptake inhibitors (SSRIs) drug (citalopram, escitalopram, dapoxetine, paroxetine, venlafaxine), and tramadol and pindolol in delaying ejaculation in patients with premature ejaculation (PE).
Materials and Methods: A predetermined number of married men with PE were randomly assigned to receive the study drug or placebo. Pretreatment evaluation included history and physical examination, intravaginal ejaculatory latency time (IELT), International Index of Erectile Function (IIEF) and Meares-Stamey test. The efficacy of each treatment was assessed every 2 weeks during treatment, at the end of study using responses to IIEF, IELT evaluation, mean intercourse satisfaction domain, mean weekly coitus episodes and adverse drug effects.
Results: The IELT after citalopram and placebo gradually increased from 32 and 28 seconds to approximately

268 and 38 seconds, respectively. The mean IELT after dapoxetine and placebo increased from 28 and 31 seconds to approximately 193 and 54 seconds, respectively (P =0.001). At the end of trial with dapoxetine, paroxetine, and placebo the mean IELT was increased from 38, 31 and 34 seconds to 179, 370 and 55 seconds, respectively. The mean IELT increased from 31 and 29 seconds to 516 and 54 seconds with escitalopram and placebo, respectively (P=0.001). The geometric mean IELT in paroxetine-pindolol and paroxetine-placebo group demonstrated 3.7 (95% confidence interval (CI): 2.16-5.26) and 1.7 (95% CI: 0.82-1.81) fold-increase, respectively (P=0.001). The mean IELT after tramadol and placebo increased from 19 and 21 seconds to approximately 243 and 34 seconds, respectively (P <0.001). The geometric mean IELT in venlafaxine and placebo group demonstrated 1.7 (95% CI; 0.76-1.96) and 1.6 (95% CI: 0.87-1.84) fold-increase, respectively (P=0.1).
Conclusion: Oral citalopram and escitalopram is a highly effective treatment for PE with long-term benefit for the patient after it is withdrawn. Paroxetine appears to provide significantly better results in terms of IELT and intercourse satisfaction versus dapoxetine, and tramadol. Venlafaxine is no better than placebo in treatment of PE. A single high dose of pindolol (7.5 mg) is an effective augmentation strategy in paroxetine-refractory patients.

Keywords: Premature Ejaculation, Treatment, SSRI, Serotonin, Sexual Dysfunction

O-6: Effect of Varicocele on Chromatin Condensation and DNA Integrity of Ejaculated Spermatozoa Using Cytochemical Tests

Talebi AR*, Moein MR, Tabibnejad N, Ghasemzadeh J

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

Objective: Varicocele occurs in approximately 15% to 20% of the general male population and it is the most common cause of poor semen production and decreased semen quality. It has been demonstrated that patients with varicocele have a significantly higher DNA fragmentation index (DFI) and spermatozoa with nuclear anomalies than healthy fertile men. Therefore, the aim of this study was to evaluate sperm chromatin integrity in these patients.
Materials and Methods: Sixty men referring to the andrology laboratory were categorised into three different groups: 20 infertile men with varicocele, 20 infertile men with abnormal semen parameters and 20 fertile men who had normal spermatogram were considered as control group. Semen analysis was performed according to WHO criteria. To evaluate sperm chromatin quality and DNA integrity, after fixation of sperm smears, aniline blue, toluidine blue, chromomycin A3 and acridine orange staining were applied in three groups. The slides were analysed by light and fluorescent microscopy and to determine the percentage of mature or immature spermatozoa, 200 spermatozoa were counted in each slide.
Results: The results showed that the rates of aniline blue-reacted spermatozoa were significantly higher in infertile and varicocele patients than in the normal group ($p < 0.001$). In addition, with regard to chromomycin A3, acridine orange and toluidine blue staining, there was a significant difference between the three groups ($p < 0.001$). The results showed that the varicocele samples contain a higher proportion of spermatozoa with abnormal DNA and immature chromatin than those from fertile men as well as infertile men without varicocele.
Conclusion: Therefore, varicocele results in the production of spermatozoa with less condensed chromatin and this is one of the possible causes of infertility due to varicocele.

Keywords: Chromatin Condensation, DNA Integrity, Sperm, Varicocele

O-7: Impact of Mature and Immature Sertoli Cells on Mouse Spermatogonial Stem Cells Proliferation

Tavakolifar F[1, 2]*, Shahverdi A[1], Pirouz M[1], Koruji M[3], Shakeri M[4], Baharvand H[1, 2]

1. Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cells Biology and Technology, ACECR, Tehran, Iran
2. Developmental Biology, Science and Culture Univercity, Tehran, Iran
3. Anatomical Science, IUMS, Tehran, Iran
4. Agricultural Campus, University of Tehran, Tehran, Iran
Email: tavakolifar_516@yahoo.com

Objective: Spermatogonial stem cells (SSCs) are unique population of adult stem cells in mammalian testes which continuously provide gametes and transfer genetic material to the next generation. Various feeder layers have been tested to support the *in vitro* culture of SSCs; however, age effect of feeder cells has been remained a controversial issue. This study was initiated to compare Sertoli cells derived from neonatal and adult mice to examine age effect of Sertoli cells in the maintenance and proliferation of mouse SSCs *in vitro*.
Materials and Methods: SSCs were isolated from testes of 6 day-old mice and culture *in vitro* at the presence of Glial-derived neurotrophic factor (GDNF) for 10 days and then transferred to Sertoli cells isolated by DSA lectin from neonatal (6 day-old) and adult (6-8 week-old) mice. After 5 days, area and number of SSC colonies were measured. Immunostaining was used to detect expression of spermatogonial markers including α6/ß1-Integrin, C-kit and Oct-4. In addition, SSC colonies were harvested at day 5 and the percentage of α6/ß1-Integrin-positive cells was measured by flowcytometery. Transplantation assay was used to confirm the

stemness of spermatogonial cells.
Results: Immunostaining analysis showed that our culture system contained SSC colonies as they were positive for α6-Integrin, ß1-Integrin and Oct-4 and negative for C-kit. In addition, these stem cells were functional as they were able to migrate to seminiferous basal membrane after transplantation to testes of busulfan-induced infertile adult mice,Results showed 5 days after co-culture of SSCs with Sertoli cells, the area and number of colonies and number of cells in each colon were significantly higher on Immature Sertoli cells. Moreover, results showed that colony efficiency of cultured SSCs on Immature Sertoli cells was significantly higher than other two groups. Flowcytometry analysis revealed that there was significant increase in the number of α6-Integrin-positive cells and ß1-Integrin-positive cells in the culture with Immature Sertoli cells (72.9 ± 14.9 %) (67.4 ± 7.7) in contrast to culture with Mature Sertoli cells (29.5 ± 6.5 %)(52.9± 1.9).
Conclusion: In conclusion , the number of SSCs was higher in co-culture with Immature Sertoli cells. It could be referred to difference in microenvironments that Immature and Mature Sertoli cells provide for *in vitro* culture of SSCs. As SSCs was derived from neonatal mice, another interpretation is that more suitable culture condition could be obtained when SSCs and feeder Sertoli cells are derived from mice in the same age; either from neonatal mice or from adult ones. Further studies would confirm these hypotheses.

Keywords: Spermatogonial Stem Cells, Sertoli Cells, Testis, Transplantation

Embryology

O-8: Can Fresh Embryo Transfers be Replaced by Cryopreserved-Thawed Embryo Transfers in Assisted Reproductive Cycles? A Randomized Controlled Trial

Aflatoonian A*, Oskouian H, Ahmadi S

Research and Clinical Center for Infertility, Tehran, Iran
Emai: homaoskouian@gmail.com

Objective: Controlled ovarian stimulation (COS) has been shown to advance endometrial maturation and affect adversely implantation in assisted reproduction technology (ART) cycles. It has been reported that there is a better embryo-endometrium synchrony in frozen-thawed embryo transfer (FET) cycles than fresh embryo transfer (ET) cycles. The objective of this study was to compare ongoing pregnancy rate between fresh ET and FET cycles in ART.
Materials and Methods: In a prospective, controlled study, the patients who were classified as high responders, were randomized to either fresh ET or FET. The embryos in FET group were cryopreserved with vitrification by Cryotop method. Randomization was done on the day of ET according to a computer-generated random numbers. Ongoing pregnancy rate was the primary outcome measure.
Results: A total of 374 patients were included, 187 of which were randomized to FET and 187 to fresh ET. There were 39% (n= 73) ongoing pregnancy in FET group compared with 27.8% (n= 52) in fresh ET group [odds ratio (OR) = 1.66; 95% confidence interval (CI) = 1.07-2.56; $p < 0.05$]. Implantation, clinical pregnancy and multiple pregnancy rates were also higher in FET group.
Conclusion: FETs can be performed instead of fresh ETs to improve the outcome of ART cycles in highly selected patients.

Keywords: Fresh Embryo Transfer, Frozen-Thawed Embryo Transfer, Vitrification, Endometrial Receptivity, Ongoing Pregnancy

O-9: A Low Intake of Antioxidant Nutrients Is Associated with Poor Semen Quality in Patients Attending Fertility Clinics

Akhbardeh M

Washington University WSPA, Washington, USA
Email: mahdi_akhbardeh14@yahoo.com

Objective: To compare specific nutrient intake between normospermic and oligoasthenoteratospermic patients attending infertility clinics in two Mediterranean provinces of Spain
Materials and Methods: Case-control study. Setting: Private fertility clinics in southeastern Spain. Patient(s): Thirty men with poor semen quality (case subjects) and 31 normospermic control subjects of couples attending our fertility clinics. Intervention(s): We recorded dietary habits and nutrient consumption using a food frequency questionnaire adapted to meet specific study objectives. Main Outcome Measure(s): We calculated nutrient intakes by multiplying the frequency of use for each food by the nutrient composition of the portion size specified on the food frequency questionnaire and by addition across all foods to obtain a total nutrient intake for each individual. Semen quality was assessed by measuring volume, concentration, motility, and morphology. Hormones levels were also analyzed in case and control subjects.
Results: In the logistic regression, control subjects had a significantly higher intake of carbohydrates, fiber, folate, vitamin C, and lycopene and lower intakes of proteins and total fat
Conclusion: A low intake of antioxidant nutrients was associated with a poor semen quality in this case-control study of Spanish men attending infertility clinics

Keywords: Food Frequency, Male Infertility, Antioxidants, Vitamins Several

O-10: Effect of Fibroblastic Growth Factor on Resumption of Meiosis, *In Vitro* Maturation and Embryo Development of Immature Mouse Oocytes

Azarnia M[1]*, Ghasemian F[1], Bahadori MH[2], Nasiri E[2], Ghasemi F[2]

1. Tarbiat Moallem University, Tehran, Iran
2. Anatomy Department, Guilan University of Medical Sciences, Rasht, Iran
Email: yasaman_21_a@yahoo.com

Objective: The purpose of this study was to evaluated the effect of fibroblastic growth factor on resumption of meiosis, *in vitro* maturation of immature mouse oocytes and resulting embryo development with and without FGF
Materials and Methods: Cumulus – oocyte complex (COCs) and germinal vasicle (GV) were obtained from female NMRI mice 46-48 hours after administration of an i.p. injection of 5 IU PMSG. COCs were released from larg antral follicles and culture for 18 hours in humidified atmosphere with 5% CO_2 at 37C° in TCM199 supplemented with 0,10,20,50 and 100ng/ml FGF. After *in vitro* maturation (IVM), metaphase II(MII)oocytes were co-incubated with sperms for 4 hours in T6 medium. For all groups, 2PN embryos were cultured in the same medium and cleaved embryos was assessed after 48 hours.
Results: FGF increased the proportion of *in vitro* growing (IVG) oocytes reached metaphase II in all compared rate, recorded in the 20 ng/ml FGF treatment groups (42.8%), was significantly higher ($p<0.05$) than the 10 ng/ml (31.1%), 50 ng/ml(9.5%) and 100 ng/ml FGF(6.6%) containing treatment groups but no significant difference was found as compared to control(29%).
Conclusion: Exogenous FGF during IVM improved the nuclear maturation and embryo development.

Keywords: Fibroblast Growth Factor, Mouse, Oocyte Maturation

O-11: Characteristics of Amniotic Fluid Cells *In Vitro* and Attempts to Improve Culture Techniques: A Mouse Model

Melina Babaee[1]*, Kamran Mansouri[1], Ali Amini[2]

1. Medical Biology Research Center, Kermanshah, Iran
2. University of New South Wales (UNSW), Sydney, Australia
Email: ml_bae_bio_bsc@yahoo.com

O**bjective:** Amniocentesis is the most common test used for prenatal diagnosis of a chromosome problem in the fetus. It involves the removing a small amount of amniotic fluid which surrounds the fetus in the amniotic sac. The amniocentesis sample is sent to the laboratory where the cells are cultured and then the chromosomes are analyzed. A new supplemented medium has been developed to improve amniotic fluid cell growth in mouse.
Materials and Methods: The medium consists of a mixture of Ham's F12 medium and Dulbecco's modified Eagle's medium (DMEM) supplemented with antibiotics, and 20% fetal calf serum (FCS).
Results: Good clonal growth is achieved consistently in 4-6 days without any mycoplasma contamination. The volume of amniotic fluid required to initiate culture can be as little as 1 ml. Amniotic fluid samples contaminated with red blood cells with no visible clot also grow well.
Conclusion: The results of amniocentesis will indicate the likelihood of the fetus developing certain chromosomal conditions, such as Down's syndrome, Edward's syndrome, and Patau's syndrome, which are all conditions where the fetus is born with an extra chromosome.

Keywords: Amniocentesis, Primary Amniotic Fluid Cell Culture, Amniotic Fluid, Bloody Taps, Animal Model

O-12: Periodic Activation of Wnt/beta-Catenin Signaling Enhances Somatic Cell Reprogramming Mediated by Cell Fusion

Cosma M.P*, Lluis F, Pedone E, Pepe

TIGEM, Telethon Institute of Genetics and Medicine, Naples, Italy
Email: cosma@tigem.it

Objective: Reprogramming of nuclei allows the dedifferentiation of differentiated cells. Somatic cells can undergo epigenetic modifications and reprogramming through their fusion with embryonic stem cells (ESCs) or after overexpression of a specific blend of ESC transcription factor-encoding genes. Our goal was to demonstrate that the activation of Wnt/beta-catenin signaling triggered somatic cell reprogramming.
Materials and Methods: We performed cell fusion experiments by fusing ES cells with Neural stem cells or ES cells with Thymocytes or ES cells with Mouse Embryonic Fibroblasts. ES cells or the hybrids were treated with Wnt3a or BIO (Wnt pathway activator) or Dkk1 (Wnt pathway inhibitor). Fusion between ES cells overexpressing different amounts of beta-catenin with NS cells were also carried out. Reprogrammed cells were analyzed in vivo and *in vitro* for their ability to differentiate in different cell types. Expression of stem cell markers and methylation profile of stem cell promoters was also analyzed in the reprogrammed cells. For further details see Lluis et al. Cell Stem Cell 2008.
Results: We showed that cyclic activation of Wnt/beta-catenin signaling in ESCs with Wnt3a or the glycogen synthase kinase-3 (GSK-3) inhibitor 6-bromoindirubin-3'-oxime (BIO) strikingly enhanced the ability of ESCs to reprogram somatic cells after fusion. In addition, we showed that reprogramming is triggered by a dose-dependent accumulation of active beta-catenin. Repro-

grammed clones expressed ESC-specific genes, silenced somatic differentiation markers, became demethylated on Oct4 and Nanog CpG islands, and were able to differentiate into cardiomyocytes *in vitro* and to generate teratomas in vivo.
Conclusion: Our data thus demonstrate that in ESCs, periodic beta-catenin accumulation via the Wnt/beta-catenin pathway provides a specific threshold that leads to the reprogramming of somatic cells after fusion.

Keywords: Reprogramming, ES cells, Wnt/beta-Catenin Signaling Pathway, Cell Fusion

O-13: Expression Profiles of Genes Controlling Maturation of Sheep Cumulus Oocyte Complexes follow Vitrification

Ebrahimi B[1]*, Rezazadeh Valojerdi M[1, 2], Eftekhari Yazdi P[2], Baharvand H[3]

1. Department of Anatomy, Tarbiat Modares University, Tehran, Iran
2. Department of Embryology, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Stem Cells, Royan Institute, Tehran, Iran
Email: gokind2004@yahoo.com

Objective: This study was conducted to compare the expression of maturation genes of sheep cumulus-oocyte complexes follow vitrification by conventional and cryotop methods.
Materials and Methods: cumulus-oocyte complexes (COCs) were harvested from slaughtered sheep ovaries. COCs were divided into three groups: Control, Conventional Vitrification & Cryotop Vitrification. In control group, COCs were transferred immediately to *in vitro* maturation medium (IVM). Vitrification was done by DMSO& EG. The viability of vitrified-warmed COCs was assessed morphologically. Vitrified-warmed COCs were matured like the control group and oocytes nuclear stage was determined by Hoechst staining. Oocytes were subjected for assessment of maturation genes expression by Real-Time quantitative RT-PCR.
Results: Cryotop vitrification had higher percent of healthy COCs after warming (83.84%) and also showed a significant difference with conventional vitrification. Mature oocytes (MII) were 51.94% and 48.81% in the control and cryotop groups respectively. Conventional vitrification showed a significant difference with control group in the expression of GDF9, BMP15 & BMPRII genes, also GDF9 & BMP15 in the conventional vitrification had a significant difference with cryotop vitrification. The relative expression of BMP15 & BMPRII was significantly different between control and cryotop groups. ALK5 expression was evaluated too.
Conclusion: According to the less success of immature sheep oocytes cryopreservation, it seems that vitrification by cryotop can reduce cryoinjuries and increase the viability, post-thaw quality and maturation rate of COCs. This kind of vitrification causes the least expression changes of maturation genes in comparison with conventional vitrification.

Keywords: Vitrification, Cryotop, Maturation Gene, Sheep, Immature Oocyte

O-14: Comparison of Two Different Methods of Dehydration for Vitrification of Sheep Ovarian Tissue

Fathi R*, Rezazadeh Valojerdi M, Hasani F

Department of Embryology, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: rfathi79@gmail.com

Objective: The aim of this study was to evaluate the effect of vitrification with two methods of 4 and 2-step dehydration on sheep ovarian tissue.
Materials and Methods: Sheep ovarian tissue samples were collected and allocated to three groups of fresh, 4-step vitrified and 2-step vitrified. A modified carrier and vitrification solution was applied. The proportion of morphologically intact follicles in fresh ovarian tissues was compared with that in vitrified-warmed tissues. The base medium (BM) containing hepessed tissue culture medium (HTCM) supplemented with 10% human serum albumin (HSA) was used as the solvents for vitrification and warming solutions. The ovarian tissue pieces were dehydrated by using regimens; 4-step: (1) 3.75% ethylene glycol (EG) and dimethyl sulfoxide (DMSO); (2) 7.5 % EG and DMSO; (3) 10% EG and DMSO; (4) 15% EG and DMSO + 0.25 mM sucrose in the base medium each for 5 minutes at 4°C and 2-step: (1) 7.5% EG and DMSO; (2) 15% EG and DMSO + 0.25 mM sucrose in the base medium each for 10 minutes at 4°C. Also imaging of apoptotic follicles was performed in fresh and vitrified tissues using TUNEL protocol.
Results: The proportion of intact antral follicles in the fresh (66.66% ± 0.33) and 2-step vitrified (56.66% ± 0.16) groups were significantly higher than that in the 4-step vitrified (0%) group whereas the difference of this proportion between fresh and 2-step vitrified was not significant But between the three groups in the proportion of primordial, primary and preantral follicles the differences was not statistically significant. The apoptosis imaging of ovarian pieces also did not show statistically differences between all the groups.
Conclusion: These results indicated that sheep ovarian tissue vitrification by 2-step method is simpler and more effective than those of 4-step method. On the other hand, as sheep and human ovarian tissue are more similar, this technique can be exam for cryopreservation of the human ovarian tissue too.

Keywords: Sheep, Ovary, Vitrification

O-15: Relationship between Follicle Growth and Circulating Gonadotrophin Levels during Postna-

tal Development of Sheep

Mahdi D[1]*, Khallili K[2], Dhimi A[3]

1. Animal Biology and Physiology, Larbi Ben M'Hidi, Oum El Bouaghi, Algeria
2. Animal Biology, Badji Mokhtar, Annaba, Algeria
3. Elevage, Institut Technique D'elevage D'ovins, Ain Mlila, Algeria
Email: dmahdij@yahoo.com

Objective: This study investigates the number and size of ovarian antral follicles in relation to plasma follicle stimulating hormones (FSH) and luteinizing hormone (LH) concentrations from birth to 26 weeks of age in ewe lambs of the Ouled Djellel breed, a non-seasonal breed of sheep.
Materials and Methods: Plasma was collected from 10 ewe lambs at 14 sampling times (Week 0, i.e. <24 h, Week 1 and every two weeks from Week 4 to Week 26, inclusive). At each of these stages, four ewe lambs were slaughtered, the ovaries recovered and weighed, and the number and size of the follicles determined from histological examination.
Results: The pattern for plasma FSH showed a peak at Week 10, a smaller peak at Week 18 and a very small peak at Week 24. The pattern for LH was similar until Week 24 when the largest peak occurred. Paired ovarian weight increased rapidly from birth to four weeks and then more slowly to 10 weeks, followed by a decline at 12 weeks and a gradual increase from 14 to 24 weeks of age. The number and total diameter of follicles ≥3 mm in diameter showed similar patterns of development – rising gradually from birth to week 14, falling to week 16 and then rising more rapidly to a peak at week 24. Maximum follicle diameter declined from birth to Week 1, then rose rapidly to Week 4, followed by a more gradual rise to week 14 and, thereafter, a more rapid increase to a peak of 7.23 ± 0.16mm at 24 weeks old. The number of follicles (<3mm diameter) increased rapidly from birth to week 10 and then declined to values similar to those at weeks 1 and 4. First behavioural oestrus was observed at week 24 and a corpus luteum was present on the ovary of one lamb at Week 24 and two lambs at week 26.
Conclusion: It was concluded that two or three peaks in plasma FSH and LH levels precede puberty and first and the increase in follicle numbers and size generally reflected the pattern of plasma FSH and LH levels.

Keywords: Ewe Lamb, FSH, LH, Antral Follicles, Postnatal Development, Ouled Djellel

O-16: gp130 Gene Expression during *In Vitro* Maturation of Mouse Oocyte

Mohammadi Roshandeh A[1]*, Haji Hossenlou H[1], Halabian R[2], Habibi Roudkenar H[2], Ebrahimi M[2], Niknafs B[1]

1. Anatomical Sciences Department, Medicine Faculty, Medical University of Tabriz, Tabriz, Iran
2. Research Center, Iranian Blood Transfusion Organization, Tehran, Iran
Email: dinachal@yahoo.com

Objective: LIF is a 45-56 kDa glycoprotein that has important role in proliferation and embryo implantation. LIF affcts its function via its receptors, such as gp130. Effects of LIF in oocyte matuiration and expression of its receptor in oocytes is unknown.
Materials and Methods: Immature mice superovulated with HMG and GV oocytes obtaind from ovary 48 hours after. The GV oocytes were cultured in TCM199 with 5% Co2 for obtaining MII oocyte. For Real-time RT-PCR, Total RNA from GV and MII oocytes were 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 gp130 was determined by Real time RT-PCR.
Results: Our results showed that gp130 is expressed neither in GV nor in MII oocytes during *in vitro* maturation of mouse oocytes. It is proven that gp130 is expressed in human oocyte but is not expressed in mouse oocytes.
Conclusion: It is suggested that in mouse, LIF could affects the oocyte via another receptor. However its details must be elucidated.

Keywords: IVM, LIF, gp 130, Oocyte, Mouse

O-17: The Effect of Hydrostatic Pressure on *In Vitro* Maturation of Oocytes Derived from the Stimulated and Unstimulated Ovary

Momeni M*, Azadbakht M, Amini A

Biology, Razi University, Kermanshah, Iran
Email: momeni.m60@gmail.com

Objective: Oocyte maturation is a process that during which the oocyte attains the competence to be fertilized. For clinical application, most oocytes are obtained from ovarian follicles of women who have been treated with hormones. Side effects of superovulation are ovarian hyperstimulation syndrome or polycystic ovary syndrome. Hence, there is an increasing interest in retrieving oocytes without gonadotropins and then *in vitro* maturation. Hydrostatic pressure as a physical force is effective on reproductive system. Obviously, there is an increase in intrafollicular pressure 15-20 mmHg in the ovulating follicle during the late stage of the ovulatory process. In this study, we examined the effect of hydrostatic pressure on *in vitro* maturation of oocytes derived from the stimulated and unstimulated ovary.
Materials and Methods: In this study we had two experimental groups. In experiment I, female NMRI mice 6-8-week-old received 10 IU/ml PMSG hormone and in

experiment II did not. Preovulatory follicles were isolated from mice ovaries, each follicle cultured individually in microdrops of MEM-α supplemented with 5% fetal bovine serum, 100-mIU/ml rFSH(Gonal-f), 10ng/ml recombinant Epidermal Growth Factor, 7.5 mIU/ml HCG under mineral oil for maturation. At the start of *in vitro* maturation follicles from two experiments divided into two treatments. In treatment I follicles were transferred to pressure chamber and exposed to 20 mmHg hydrostatic pressures for 30 min and in treatment II follicles were transferred to pressure chamber without hydrostatic pressure exposure. Then follicles were cultured for maturation of oocyte.

Results: After 24 h in experiment I percent of MII oocyte was increased in follicles with hydrostatic pressure exposure (15.38%) compared to follicles without hydrostatic pressure exposure (10%) ($P<0.05$). In experiment II percent of MII oocyte was increased in follicles with hydrostatic pressure exposure (18.42%) compared to follicles without hydrostatic pressure exposure (10.52%)($P<0.05$). After 48 h in experiment I, percent of MII oocyte was increased in follicles with hydrostatic pressure exposure (38.46%) compared to follicles without hydrostatic pressure exposure (20%)($P<0.05$). In experiment II percent of MII oocyte was same in follicles with hydrostatic pressure exposure (44.8%) and in follicles without hydrostatic pressure exposure (42.1%).

Conclusion: Gonadotropin stimulation is used to superovulation, achieves multifollicular recruitment, but reduces oocyte *in vitro* maturation. According to our findings hydrostatic pressure can be improve oocyte *in vitro* maturation by positive effect on the preovulatory follicle.

Keywords: Hydrostatic Pressure, Hyperstimulation, Oocyte Maturation, Mouse

O-18: The Effect of Intercourse on Pregnancy Rate in IVF Cycles, A Preliminary Report

Naghibi Harat Z[1, 2*], Karimian L[1], Yazdi P.E[1], Moini A[2, 3], Valojerdi M.R[1], Akhondi M.M[1], Shahverdi H[1], Movaghar B[1], Hassani F[1], Sedighi-Gilani M.A[4]

1. Department of Embryology, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Rooin-Tan Arash Hospital, Tehran Medical University, Tehran, Iran
3. Department of Endocrinology and Femail Infertility, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
4. Department of Andrology, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: rezaiemj@hotmail.com

Objective: Positive effects of sperm and seminal plasma on female genital tract, embryo vitality and implantation had been explained in some animal and human experiments. But there is a doubt to advise intercourse or abstinence in infertile couples during the in-vitro fertilization (IVF) cycles. We designed a prospective controlled trial to find out the intercourse effect on intra cytoplasmic sperm injection (ICSI) cycle outcome among of 1321 fresh embryo transfer. Here we report the results of pregnancy and implantation rate in 225 cycles of carrying 496 embryos which had been included till now.

Materials and Methods: A total of 225 patients undergoing IVF/ICSI treatment between October and December of 2008 were randomly allocated into the case (107 people) and the control group (105 people). It was explained in case group to have intercourse 12 to 24 hours before ovum pickup and 12 hours after embryo transfer. Control group were forbidden to have intercourse in 72 hours before until 2 weeks after embryo transfer. All embryos were cultured and transferred in the same procedures. Chemical pregnancy was established in 14 and 17days after transfer by measuring βHCG level in plasma and clinical pregnancy in 6 weeks after transfer by appearance of gestational sac in ultrasonography.

Results: Among 225 patients who were participated in trial, 13 cycles resulted to no embryo transfer (5.8%). Mean ages of couples, duration and cause of infertility, number of oocyte, fertilization rate, source of injected sperm and sperm parameters in each group were the same.

496 embryos in 212 people were transferred totally which resulted in 64 pregnancies (30.1%). Pregnancy rate in case group was 31.8% versus 28.6% in control group; the difference was not statistically significant. Both βHCG level differences were significant between groups ($p<0.05$). Number of gestational sac was the same in both groups.

Conclusion: This study supports the findings of other similar experiments which indicate that semen could enhance the endometrium and promoting the pregnancy. Although the pregnancy rate was the same in this preliminary analysis but the higher βHCG level in case group was significant. It suggests that intercourse may probably make a better chance for pregnancy but of course it is necessary to study a large number of patients. At this time we can suggest that there is not necessary to advise all patients to have abstinence during IVF cycle.

Keywords: Intercourse, ART Outcome, Abstinence

O-19: Relationship of Sperm Morphological Abnormalities with Levels of Reactive Oxygen Species in Semen Specimens

Nasr Esfahani M*, Ghasemzade J, Razavi Sh
Shahid Sdoughi University of Yazd, Yazd, Iran
Email: dr.matin.nasr@gmail.com

Objective: we compared ROS levels in seminal plasma of infertile men with this level in healthy donors. We also determined the ROS level association with percentages of different sperm morphological abnormalities, the sperm deformity index, and the teratozoospermic index scores.

Materials and Methods: In total 30 infertile patients

and 25 healthy donors as control were selected. Sperm analysis was done for all patients. Azoospermic patients were excluded from the study. ROS level in semen were measured by a chemiluminescence assay in both group. Thin smears af well-mixed semen were stained with papanicolaou to assess morphological abnormalities.
Results: The mean ROS level in normal men was 166.10 RLU (Relative Light Unit) while this was 1630.50 RLU in infertile patient which is significantly higher in case group ($p = 0.000$). A significant positive correlation was observed between sperm ROS production and the proportion of sperm with abnormal morphology. ROS production was positively correlated with the proportion of sperm with amorphous head, midpiece defects, cytoplasmic droplets, tail defect, pinhead sperms, SDI scores, and TZI scores ($p<0.005$)
Conclusion: The level of ROS in seminal fluid of infertile men is significantly higher than fertile donors and also sperm morphological abnormalities, SDI score and TZI score are useful to predict levels of ROS.

Keywords: Male Infertility, Reactive Oxygen Species (ROS), Sperm Morphology

O-20: The Leading Blastomere of the 2-Cell Stage Parthenogenetic Porcine Embryo Contributes to the Abembryonic Part First

Roh S

School of Dentistry, Seoul National University, Seoul, Korea
Email: sangho@snu.ac.kr

Objective: Here in, we investigated the embryonic-abembryonic axis of the blastocyst in the porcine species. To avoid the influences of the fertilization cone, which indicates the sperm entry position, and to prevent topological change of the two or more apposing pronuclei in the egg center caused by polyspermy after IVF, we chose porcine parthenogenetic embryos for use in the present study even though they may not represent normal embryonic development in the pig. Here in, we describe the fate of an individual blastomere from a 2-cell-stage parthenogenetic porcine embryo.
Materials and Methods: For lineage tracing, DiI, a fluorescence dye, was injected into only a blastomere of the 2-cell stage parthenogenetic porcine embryos. If the first blastomere to divide was labeled, the embryo was included in the leading group, and while all others were included in the lagging group. Mitochondrial distribution of 2-cell parthenotes was also examined by using MitoTracker Green staining and confocal system.
Results: In 60.5% of the blastocysts in the lagging group, the progeny of the labeled blastomeres formed the inner cell mass (ICM) and adjacent trophectoderm (TE) hemisphere; 62.1% of the blastocysts in the leading group had progeny of the labeled blastomeres distributed only to the TE (opposite of ICM). The rest of the lagging and leading groups showed random distributions. Unlike murine parthenotes, biased mitochondrial distribution was also found in porcine parthenotes (38.1%).
Conclusion: Our findings indicate that the 'leading' blastomere of the 2-cell porcine parthenote forms the distal TE (abembryonic) and that the 'lagging' blastomere forms the remaining portion of the blastocyst, including the ICM (embryonic). Biased distribution of mitochondria in each 2-cell blastomere may contribute partly to this event. This study was supported by Korea Science and Engineering Foundation (KOSEF) grants funded by the Government of the Republic of Korea (MOST; M10641000001-06N4100-00110 and R01-2007-000-10316-0).

Keywords: Embryonic, Abembryonic Polarity, Mitochondria, Parthenogenesis, Porcine

O-21: Molecular Assessment of the Uterine Milieu during Implantation Window in Humans and Non-human Primates

Geetanjali Sachdeva, Vishakha Patil, Gracy Rosario, Tanu Parmar, Shruti Nimbkar-Joshi, Rajendra Katkam, Lalita Savardekar, Dhananjay Manjramkar, Chander Parkash Puri

Primate Biology Division, NIRRH, Indian Council of Medical Research, Mumbai, India
Email: sachdevag@nirrh.res.in

Objective: a) To identify the endometrial factors which are directly or indirectly regulated by progesterone in nonconception cycle and also to investigate whether their expression is modulated during early pregnancy in primates and b) To identify the factors which are differentially expressed in endometrial tissues and uterine secretions during the progesterone dominant or mid-secretory phase as compared to the estrogen dominant or proliferative phase in humans
Materials and Methods: For objective a, six regularly cycling healthy female bonnet monkeys (Macaca radiata) were subcutaneously injected with Onapristone- ZK 98.299, an antiprogestin, dissolved in vehicle (benzyl benzoate: castor oil, 9:1), at a dose of 5.0 mg starting from day 1 of the menstrual cycle and continued every third day for one cycle. Onapristone treatment rendered the animals infertile or implantation incompetent due to induction of endometrial nonreceptivity. Control animals (n=6) were treated with vehicle alone. Circulatory steroid levels were estimated in animals before and after the treatment using radioimmunoassays (Sachdeva et al, 2001; Patil et al, 2005). Endometrial biopsies were collected from both onapristone-treated and vehicle-treated animals on day 8 estradiol peak. Immunohistochemistry and reverse transcriptase polymerase chain reaction were used to investigate whether some of the select factors were differentially expressed after the blockade of optimal progesterone

action on endometrium in bonnet monkeys. Differential display reverse transcriptase polymerase chain reaction (DDRTPCR) and 2D proteomics approaches were also used to identify the factors which were differentially expressed in the endometrium of implantation incompetent bonnet monkeys, compared to vehicle treated control animals. To investigate whether the expression of some of these factors is altered during pregnancy, endometrial samples were collected from another group of animals on day 6 of pregnancy (approximately equivalent to day 8 post estradiol peak). Towards this, regularly cycling female bonnet monkeys (n=6) with normal hormonal profiles (peak estradiol levels- 300-600 pg/ml; progesterone levels (3-6 ng/ml) were mated with males of proven fertility for six continuous days starting from two days prior to the expected estradiol peak. Pre-implantation factor (PIF) in the sera was used as a surrogate marker of pregnancy (Rosario et al, 2005a). The control group included nine PIF negative animals. For objective b, regularly cycling women (21-35 years) of proven fertility with a history of at least one live birth were enrolled in the study. Ovulation was monitored by serial ultrasonography (USG) to ascertain the follicular collapse. Endometrial tissue and uterine fluid samples were collected from women on day 6 post-ovulation (in mid-secretory phase) or on day 2-3 prior to ovulation (in proliferative phase). 2D proteomics and immunoblot analysis were used to identify the factors, which were differentially expressed during the progesterone dominant phase as compared to the proliferative phase in human endometrial tissues and uterine fluid samples.

Results: Candidate factor approach revealed differential expression of several cytokines such as interleukin 1 beta, interleukin 6, transforming growth factor beta, leukemia inhibitory factor (Sachdeva et al, 2001) and cell adhesion molecules like alpha v and beta 3 integrin (Puri et al, 2000) in the mid-secretory phase endometria of antiprogestin treated bonnet monkeys as compared to that of control animals. This suggested that progesterone directly or indirectly regulates the expression of these factors. Interestingly, expression of interleukin 6, transforming growth factor beta in endometrium was significantly higher in the endometria of pregnant animals, as compared to that in nonpregnant animals (Rosario et al, 2005b), whereas the expression of leukemia inhibitory factor did not alter significantly during early stages of pregnancy. Integrins alpha v and beta 3 also showed cell type specific increase in endometrium during early stages of pregnancy (Nimbkar-Joshi et al, unpublished). These studies indicated that progesterone priming during nonconception cycle leads to increase in the expressions of endometrial TGF beta, LIF, interleukin 6, integrins and these factors probably facilitate endometrial preparation for implantation. Expressions of some of these factors are further modulated during early stages of pregnancy. Functional genomics approaches such as differential display RTPCR demonstrated up regulation of Rab Coupling Protein (RCP) in the endometria of antiprogestin treated animals, as compared to control animals (Patil et al, 2005). Interestingly RCP is known to be involved in the intracellular trafficking of integrins. There was no concomitant increase in the expressions of Rab4 and Rab11- proteins known to interact with RCP, suggesting impairment in the expression of specific components of intracellular trafficking pathways. These studies suggested that the blockade of progesterone action in endometrium may alter intracellular trafficking and this in turn could be responsible for the altered distribution of cell surface molecules such as integrins on endometrium. This could be one of the reasons for the incompetence of endometrium for implantation in antiprogestin treated animals. Further, 2D proteomics coupled with MALDI-TOF-TOF analysis revealed differential expression of two reticuloplasmins- endoplasmic reticulum resident proteins such as calreticulin and protein disulfide isomerase in bonnet monkeys rendered infertile with antiprogestin. Interestingly, calreticulin was also found to be less abundant in the 2D endometrial tissue protein map of mid-secretory phase as compared to that of the proliferative phase in humans (Parmar et al, 2008a). This suggested that the expression of calreticulin is downregulated in endometrial tissues during progesterone dominant phase as compared to estrogen dominant phase, Interestingly, expressions of these two proteins were also increased in endometrium during very early stages of pregnancy. It may be mentioned here that endometrial estradiol receptor alpha, an estrogen regulated gene also showed increased expression during early stages of pregnancy (Rosario et al, 2008). This suggested that the expression of these proteins is positively regulated by estradiol in vivo. Our *in vitro* studies also suggested that estradiol positively regulates the expression of calreticulin whereas progesterone is somewhat inhibitory to the expression of calreticulin. In addition to calreticulin, ? chain of fibrinogen, adenylate kinase isoenzyme 5, transferrin, annexin V, alpha-1-antitrypsin (AAT), creatine kinase and peroxidoxin 6 were also found to be differentially expressed in endometrium during the progesterone dominant phase as compared to that in the estrogen dominant phase in humans. Further similar studies on uterine fluid samples revealed higher expression of AAT and apolipoproteins during the progesterone dominant phase or mid-secretory phase as compared to that in the proliferative phase of cycle in healthy fertile regularly cycling women (Parmar et al, 2008b).

Conclusion: These studies collectively led to identification of several factors in endometrium, which are either positively or negatively regulated by progesterone. This knowledge will help in the construction of progesterone regulated functional networks, which can be targeted for contraception or for infertility management.

Keywords: Endometrium, Progesterone, Receptivity, 2D Proteomics, Uterine Fluid

O-22: Effects of Taxol on immature bovine oocyte

vitrification using Open Pulled Straw (OPS)

Shayegh M*, Barati F, Mohammadi Gh

Department of Clinical Science, Shahid Chamran University, Ahvaz, Iran
Email: baratif@scu.ac.ir

Objective: In spite of the good sperm and embryo response to cryopreservation programs, oocyte response (post-thaw maturation and fertility) to cryopreservation procedures is very poor. Special cellular status such as very sensitive cytoskeletal system, early release of cortical granules and pre-fertilization zona hardening and changes in position and structures of ZP glycoproteins are the consequences of freezing procedures on the oocytes. However, matured oocytes can tolerate the freezing procedures better than immature oocytes. Cytoskeleton stabilizer compounds, like Cytochalasin D and B, improved the vitrification outcome mature and immature oocytes. Taxol is a cytoskeleton stabilizer and used for human immature, bovine and murine mature oocyte vitrification, successfully. The aim of this study is to investigate the effects of taxol on immature bovine oocyte vitrification.
Materials and Methods: Material and Methods Indigenous bovine ovaries transported to the Lab in the physiologic saline contained penicillin streptomycin in 39oC. Cumulus Oocyte Complexes (COC) aspirated form follicles (2-8 mm) and their qualification assessed in the medium TCM supplemented with 10%FCS. Grade A oocytes subjected to five groups of experiments: Control (n=108): oocytes with the routine IVM procedure, CRP (n=51): oocytes which exposed to the vitrification solutions without vitrification and subjected to IVM procedure, Tax (n=51): oocytes which exposed to Taxol for 15 min then transferred to IVM medium, OPS (n=50): oocytes which were vitrified, thawed and subjected to IVM procedure and OPS-Taxol (n=50): oocytes which exposed to the Taxol after 15 min, vitrified, thawed and transferred to the maturation medium. IVM medium was TCM 199 supplemented with 10mg FSH, 10mg LH, 10% Bovine follicular fluid and 5% FCS. During maturation, oocytes incubated in the atmosphere with 5% CO2, 95% humidity for 24 hours. After the period of maturation, COCs denuded and stained with the conventional procedure of aceto-orcein satin for evaluating nuclear maturation. Before vitrification, COCs exposed to three medium: HM: TCM 199+10% FCS, V1: HM+10% EG+ 10% DMSO and V2: HM+20% EG+ 20% DMSO+0.05m sucrose, for 5 min within each medium then loaded in the Open Pulled Straw (OPS) and plunged into the liquid nitrogen tank. At least after 48 hours of vitrification COCs thawed in the thawing media composed of T1: HM+0.25m sucrose and T2: HM+0.15m sucrose. COCs held within each media for 5 min and transferred to the IVM medium, finally. Taxol (Abetaxel®,) with the maturation medium with dose of 1 nmol/ml. Percentage of matured oocytes were analysed with General Linear Model of SAS and means separated with the Tukey multiple comparison test. Data expressed as Least square means with SEM.
Results and Discussion: The percentage of matured oocyte was not different ($p>0.05$) between CRP (71.9 ± 5.4) and Tax (73.1 ± 6.8) groups compare to Control (80.5 ± 6.8). Vitrification (9.1 ± 6.2) significantly affected the maturation rate compare to control ($p<0.05$). The percentages of matured oocytes was higher (p.0>05) in Taxol treated vitrified oocytes compare to the oocytes which were not treated with Taxol.
Conclusion: These results show the impact of vitrification on oocyte maturation of immature bovine oocytes with no significant improvement with pre-freeze Taxol treatment.

Keyword: Oocyte Vitrification, Taxol, Nuclear Maturation, Bovine

O-23: Back Muscle as a Promising Site for Ovarian Tissue Transplantation, an Animal Model

Soleimani R[1]*, Van der Elst J[1, 4], Heytens E[1], Van den Broecke R[2], Gerris J[1], Dhont M[1], Cuvelier C[3], DE Sutter P[1]

1. Department of Reproductive Medicine, Gent University Hospital, Gent, Belgium
2. Gynecological Oncology, Department of Obstetrics and Gynecology, Ghent University Hospital, Gent, Belgium
3. Department of Pathology, Ghent University Hospital, Gent, Belgium
4. Centre for Reproductive Medicine, Universitair Ziekenhuis Brussel, Brussels, Belgium
Email: r_s902@yahoo.com

Objective: The aim of this study was to evaluate the optimal transplantation site for ovarian tissue fragments in murine hosts. We compared the transplantation to the back muscle (B) versus the kidney capsule (K) in a mouse allograft model.
Materials and Methods: Hemi-ovaries from 12-day-old mice were allografted into B and K of bilaterally ovariectomized same strain recipients which had undergone gonadotrophin stimulation (n 5 15). Graft survival after 27 days, angiogenesis and follicle development were scored and compared to age-matched control ovaries (38-day old, n 5 5). The ability of oocytes to be fertilized was studied after IVF, ICSI and embryos were transferred to recipient mothers. Anti-mouse CD 311 antibody was used to evaluate neo-vascularization in grafts.
Results: Primordial follicle survival was higher ($p < 0.01$) and vascular support was better ($p < 0.01$) in B- than in K-grafts. From 34 oocytes retrieved from B-grafts (15 metaphase I, of which 14 matured *in vitro*, and 19 collected at metaphase II), 18 morulae were obtained. Transfer of 12 embryos obtained by ICSI led to three live offspring, and transfer of six IVF embryos to another recipient mother yielded four offspring, one of which was born dead and one showed placental anomalies.

Conclusion: Primordial follicle survival was higher ($p < 0.01$) and vascular support was better ($p < 0.01$) in B-than in K-grafts. From 34 oocytes retrieved from B-grafts (15 metaphase I, of which 14 matured *in vitro*, and 19 collected at metaphase II), 18 morulae were obtained. Transfer of 12 embryos obtained by ICSI led to three live offspring, and transfer of six IVF embryos to another recipient mother yielded four offspring, one of which was born dead and one showed placental anomalies.

Keywords: Back Muscle Grafting, Follicle Survival, Live Offspring, Ovarian Tissue Transplantation, Graft Neo-Vascularization

O-24: The Developing Human Ovary: ImmunoHistochemical Analysis of Germ Cell-Specific VASA Protein, BCL2/BAX Expression Balance and Apoptosis

Vitullo A.D*, Albamonte M.S, Willis M.A, Albamonte M.I, Jensen C.F, Espinosa M.B

Centro de Estudios Biomédicos, Biotec, Ambientales y Diagnóstico, CEBBAD, Universidad Maimónides, Buenos Aires, Argentina
Email: vitullo.alfredo@maimonides.edu

Objective: Germ cell number during ovarian organogenesis is regulated through programmed cell death. We investigated the expression of germ cell-specific VASA protein, apoptosis-related proteins BAX and BCL2, and DNA fragmentation in human developing ovaries from gestation week 12 to term
Materials and Methods: Human foetal ovaries from 13 women undergoing spontaneous abortion were fixed, paraffin-embedded and processed for immunohistochemistry to analyse temporal and cellular localisation of VASA, BCL-2 and BAX, and to detect apoptosis by TUNEL assay.
Results: VASA showed a differential pattern of expression throughout differentiation and proliferative phase, prophase I to finally associate to Balbiani's body in primordial and primary follicles. BCL-2 was detected from week 12 to 17 and became undetectable thereafter. Strong BAX signal was detected in oogonia and oocytes from week 12 to term. Low levels (≤10%) of TUNEL positive germ cells were detectable throughout gestation with a higher incidence (around 20%) at 18-20 weeks.
Conclusion: VASA was specifically expressed in germ cells and displayed a stage-specific intracellular localisation enabling to follow oogenesis throughout gestation. Apoptosis-inhibiting BCL-2 was associated to germ cell proliferative phase and prophase I while BAX remained positive throughout gestation. The highest incidence of apoptotic germ cells was coincident with the lack of detectable BCL-2 protein, and when primordial follicle formation became widespread.

Keywords: Human Foetal Ovary, Germ Cells, Primordial Follicles, Apoptosis, VASA, BCL-2, BAX, Balbiani's Vitelline Space

Epidemiology and Ethics

O-25: Which One is More Ethical: Egg Donation or Egg Sharing?

Leila Alizadeh, Reza Omani Samani*

Department of Epidemiology and Reproductive Health, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: samani@royaninstitute.org

Egg sharing or oocyte sharing has different meanings: one is usage of a donor for two recipients that the recipients share the donated oocytes and compensation expenses. But the more popular definition of oocyte sharing is that an infertile woman undergoing assisted reproductive techniques gives half of her own oocytes to a recipient in return for subsidized expenses of fertility treatment. This paper focuses on the later definition and compares this procedure with oocyte donation from ethical, religious, social and legal perspective. The key results are as follows: oocyte sharing is more acceptable upon Islam, ethically it does not put a normal and fertile young woman under risk of fertility drugs, anesthesia and operations, socially it reduces the danger of "oocyte business" like changing the donor to vender, payment, brokers, arguments, advertisements, etc… and legally there is no difference between these two procedure and if donation is acceptable, so is the sharing program. There are two major concerns about egg sharing which are as follows: 1. maybe there are some psychological effect on donors who do not succeed to have a child but the recipient does. Although this effect was not reported in many researches and donors were always happy about what they did, but with complete anonymity it can be reduced. 2. There is a concern about the health of donor who is infertile and can have some health problems and advanced age which can be afforded by definition of some criteria for the suitable egg sharers.

Keywords: Egg Donation, Egg Sharing, Religious, Ethical, Legal

O-26: Men's Lived Experince of Male Infertility

Hoseini S*, Fahami F, Ehsanpour S, Zargham A

Isfahan University of Medical Sciences, Isfahan, Iran
Email: b.s.hoseini@gmail.com

Objective: The ability to reproduce and give birth to a child is an inherent part of life for most human beings. To see the next generation grow up is described as happiness and a driving force in life; thus infertility causes sever anxiety for infertile persons. Approximately 15% of all couples in the reproductive ages are involuntarily

childless, and infertility is the third cause of divorce in Iran. The purpose of this study was to explore men's experience of male infertility

Materials and Methods: Descriptive phenomenological method was used to determine these experiences. The data was collected by deep interviewing with 10 interfile men

Results: In related with infertility phenomenon we obtained four general concepts: 1. Personal anxiety. 2. Challenge with communications. 3. Effects of believes and religion. 4. Problems associated with treatment process

Conclusion: According to our emerged result in this study, it seems that all aspects of infertile men's life are affected by infertility. Thus, designing and accomplishment of consultive and supportive programs play very important roles for giving better cares to infertile men.

Keywords: Lived Experiences, Men, Infertility

O-27: I am Grateful About All it is": Religion and Spirituality as Resources of Coping with Infertility

Latifnejad Roudsari R[1]*, Allan H.T[2], Smith P.A[2]

1. Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran
2. Health and Social Care, University of Surrey, Guildford, UK
Email: latifnejadr@mums.ac.ir

Objective: Despite the lack of empirical research on the role of spirituality in the lives of infertile women, scientific literature has identified religion and spirituality as significant coping resources throughout the life course which individuals rely on to gain control in their lives. This study explored how infertile women coped with infertility using their belief system.

Materials and Methods: In a feminist grounded theory study 30 infertile women affiliated to different denominations of Christianity (Protestantism, Catholicism, Orthodoxy) and Islam (Shia and Sunni) were interviewed. Data were collected through semi structured in-depth interviews with volunteer infertile women in one Iranian and two UK fertility clinics and analyzed using Strauss and Corbin's mode of grounded theory.

Results: Religious infertile women using religious and spiritual problem-solving strategies played an important role in promoting their psychological fitness. They used a combination of both positive (benevolent religious reappraisal, belief in spiritual support, engagement in religious rituals, belief in miracle, religious surrendering, belief in timing, religious consciousness, religious helping and seeking support from clergy) and negative religious coping strategies (demonic reappraisal, discontent with God, discontent with clergy and spiritual irrelevance). Religious participants concurrently used some non-religious coping strategies (seeking friendship, looking for medical resources, not to think about, changing lifestyle and complementary therapies) in conjunction with religious coping strategies.

Conclusion: Infertile women reported a variety of religious/spiritual coping strategies which were associated with adaptive health outcomes. Further scientific inquiry is required to investigate how religion and spirituality promote healthy adaptation to infertility and whether health professionals' attention to religious/ spiritual coping could assist infertile women to be well adapted to infertility.

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

O-28: Related Factors of Sexual and Marital Satisfaction in 45-65 Years Old Women, South of Tehran

Taavoni S[1]*, Haghani H[1], Peiravi H[1], Azadi M[2]

1. Iran University of Medical Sciences, Tehran, Iran
2. Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: staavoni14@yahoo.com

Objective: To determine the relationship between sexual satisfaction, its changes, and marital satisfaction according to personal characteristics in 45-65 years old women in south of Tehran

Materials and Methods: This is a Cross sectional study that was done in 2006-2007. The valid and reliable (test re test) questioner was used. The questioner had two main parts: 1) Personal characteristics (age, couples' age difference, education level, job, No. of children, and No. of children who lives with their parents…), 2) Sexual satisfaction, its change and marital satisfaction. (Visual scale was used) The samples used in this study consisted of 161 volunteer healthy women with healthy couples, who were chosen from the public centres of low socioeconomic district in south of Tehran. We exclude the women with University degree. Descriptive and interferential statistical method (ANOVA, Scheffe, Pearson correlation) were used. The final data were analysed and tabulated in 32 tables.

Results: Referring to personal characteristics, we found remarkable points. The highest average of marital satisfaction was in 60-65, sexual satisfaction was in 45-49, and sexual satisfaction change (decrease) was in 55-60 age groups. There was only difference between marital satisfactions according to average of age group. (ANOVA, p= 0.03) By using Scheffe test we found this difference between 45-49 and 50-54 years age group. Also we found correlation between marital satisfaction and sexual satisfaction (pearson correlation, r= +0.728) and between sexual satisfaction and its change. (r= - 0.215)

Conclusion: According to personal characteristics, we only found difference between age groups. We found during early years of post menopause, there was more marital and sexual unsatisfied group, which may be because of not still coping with the menopause situation. We suggest having more education and consultation program for this group. Also we suggest having the same study with the same goals on other socio econom-

ical group and men too, for comprising the results.

Keywords: Sexual Satisfaction, Marital Satisfaction, Low Socioeconomic District

Female Infertility

O-29: Comparison of Mild Stimulation Protocol and Conventional Protocol in IVF Outcome

Mohammad Ali Karimzadeh*, Shahnaz Ahmadi, Homa Oskouian

Yazd Research and Clinical Center for Infertility, Yazd, Iran
Email: ahmadishahnaz2005@yahoo.com

Objective: To compare the efficacy of using mild ovarian stimulation protocol and conventional stimulation protocol in IVF outcome.
Materials and Methods: we randomized 200 women with regularly menstruation who were candidate for IVF. They had undergone stimulation with clomiphene citrate, gonadotropin and GnRH antagonist or gonadotropin and GnRH agonist.
Results: There were no significant difference in mean age, cause of infertility, basal FSH, BMI, endometrial thickness on the HCG administration and clinical pregnancy rate in two groups. The number of recovered oocytes , embryos obtained, embryos transferred, , peak estradiol on the HCG administration and OHSS were significantly higher in agonist group. Significantly more patients in agonist group had embryo cryopreserved
Conclusion: The CC/ gonadotropin/ GnRH antagonist is an acceptable alternative protocol for IVF in patients with regularly menstruation.

Keywords: Clomiphene Citrate, GnRH Agonist, GnRH Antagonist, *in vitro* Fertilization, Regnancy Rate

O-30: Comparison of Mild Stimulation Protocol and Conventional Protocol in IVF Outcome

Mohammad Ali Karimzadeh*, Shahnaz Ahmadi, Homa Oskouian

Yazd Research and Clinical Center for Infertility, Yazd, Iran
Email: ahmadishahnaz2005@yahoo.com

Objective: To compare the efficacy of using mild ovarian stimulation protocol and conventional stimulation protocol in IVF outcome.
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Conclusion: The CC/ gonadotropin/ GnRH antagonist is an acceptable alternative protocol for IVF in patients with regularly menstruation.

Keywords: Clomiphene Citrate, GnRH Agonist, GnRH Antagonist, *in vitro* Fertilization, Pregnancy Rate

O-31: Increased Risk of Depression in Women with Polycystic Ovarian Syndrome: (A Case-Control Study)

Hosseini R[1]*, Hadidi M[2], Rabiee M[3]

1. Department of Endocrinology and Femail Infertility, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Internal Medicine, Qom Azad University, Qom, Iran
3. Psychology Department, Qom University of Medical Science, Qom, Iran
E.mail: rohosseini@yahoo.com

Objective: PCOS is associated with several metabolic complications. Physical appearance, menstrual abnormalities and difficulty conceiving are related to lower health-related quality of life scores in these patients. The aim of this study is to estimate the prevalence of depression in PCOS patients compared with controls.
Materials and Methods: Women with PCOS (n=55) meeting the Rotterdam criteria for this syndrome, who referred to outpatient endocrine clinic in Qom, Iran, compared with control subjects (n=55) who selected from healthy women and age matched, from Dec.2007 to Sep.2008. Beck Depression Inventory (BDI) questionnaire was filled up for participants. Blood pressure, weight, height, waist circumference were measured for all subjects. PCOS patients were divided in two subgroups: Depressed and non depressed. LH, FSH, Testosterone, DHEA-S, prolactin, fasting plasma glucose, lipid profiles were measured only in PCOS subgroups.
Results: PCOS patients had significant higher prevalence of depression compared with controls (60% vs. 23.6%).The prevalence of severe depression was 21.8% vs. 1.8% in PCOS and control respectively. Systolic blood pressure (BP), diastolic BP, body mass index and waist circumference were higher than controls ($p<0.05$). We did not find significant differences in hormonal, lipid and glucose profiles between depressed and nondepressed PCOS patients. Although the numbers of infertile women was higher in PCOS patients, but this variable had not statistically significant difference between depressed and nondepressed PCOS.
Conclusion: The present study shows that women with PCOS have significantly increased risk of depression and they should be routinely screened and adequately treated for depressive disorders.

Keywords: Polycystic Ovarian Syndrome, Depression, Beck Questionnaire

O-32: A Safety and Efficacy Study of a Resorbable Hydrogel for Reduction of Post-Operative Adhesions Following Myomectomy

Mettler L[1]*, Hucke J[2], Bojahr B[3], Tinneberg H[4], Leyland N[5], Avelar R[6]

1. Kiel University, Dept. OB/GYN, Kiel, Germany
2. Wuppertal University, Dept. OB/GYN, Wuppertal, Germany
3. MIC Day Clinic Berlin, Berlin, Germany
4. Giessen University, Dept. OB/GYN, Giessen, Germany
5. St. Joseph's Health Centre Toronto, Toronto, Canada
6. Medical Affairs, Angiotec Pharmaceurticals, Vancouver, Canada
Email: lmettler@email.uni-kiel.de

Objectives: This multicenter, randomized, single-blind study assessed the safety and efficacy of a resorbable hydrogel ('Hydrogel') for the reduction of post-operative adhesion formation following myomectomy.
Materials and Methods:
Women (n 5 71) who were undergoing laparoscopic (67.6%) or laparotomic myomectomy were randomized (2:1) to Hydrogel (sprayed over surgically treated areas prior to wound closure, n 5 48) or to control (standard care, n 5 23). Patients (38 Hydrogel, 20 control) returned 8–10 weeks later for a second look. Adhesions were graded using a modified American Fertility Society (mAFS) scoring method. The primary efficacy measure was the posterior uterus mAFS score.
Results: For Hydrogel and control patients, respectively, Mean±SD mAFS scores were 0.5 ± 1.4 and 0.0 ± 0.0 at baseline, and 1.1 ± 1.9 and 2.6 ± 2.2 at the second look. Similarly, mean changes from baseline were 0.8 ± 2.0 and 2.6 ± 2.2 (P 5 0.01); 95% confidence intervals for these mean changes were (0.16 – 1.44) and (1.64 – 3.56). Adverse events were reported by 9.6 and 17.4% of Hydrogel and control patients, respectively. No intraabdominal infections or post-operative site infections were reported.
Conclusion: This 71-patient study provides the first clinical evidence of the safety and efficacy of Hydrogel for the reduction of adhesions following myomectomy

Keywords: Adhesions, Gynecologic Surgery, Laparoscopy, Laparotomy, Polyethylene Glycol

O-33: Prediction of High Ovarian Response to Controlled Ovarian Hyperstimulation: Anti-Müllerian Hormone Versus Small Antral Follicle Count (2-6 mm)

Oskouian H[1]*, Ahmadi S[1], Oskouian L[2], Aflatoonian A[1]

1. Research and Clinical Center for Infertility, Yazd, Iran
2. Mashad Medical Science, Mashad, Iran
Email: homaoskouian@gmail.com

Objective: To evaluate the predictive value of basal serum anti-müllerian hormone level and small antral follicle count for high ovarian response to controlled ovarian hyperstimulation
Materials and Methods: A total of 159 patients were prospectively included. Basal serum anti-müllerian hormone and small antral follicle count (2-6 mm) were measured.
Results: Small antral follicle count and anti-müllerian hormone have similar predictive accuracy for high ovarian response with area under curve of 0.961 and 0.922, respectively. The sensitivity and specificity for prediction of high ovarian response were 89% and 92% for small antral follicle count and 93% and 78% for anti-müllerian hormone at the cutoff values of ≥ 16 and ≥ 34.5 pmol/l, respectively.
Conclusion: Small antral follicle count and anti-müllerian hormone are equally accurate predictors of high ovarian response and facilitate determination of the optimal strategy for controlled ovarian hyperstimulation.

Keywords: Anti-Müllerian Hormone, Embryo Quality, High Ovarian Response, Ovarian Hyperstimulation Syndrome, Small Antral Follicle Count

O-34: What Harbours the Cradle of Life? The Progesterone-Dependent Immunomodulation

Polgar B[1], Kozma N[1], Berki T[2], Szekeres-Bartho J[1], Halasz M[1]*

1. Medical Microbiology and Immunology Department, University of Pecs, Pecs, Hungary
2. Immunology Department, University of Pecs, Pecs, Hungary
Email: melindahalasz@hotmail.com

Objectives: The foeto-maternal relationship during pregnancy is controlled by a complex regulation of immuno-endocrine homeostasis where progesterone-dependent immunmodulation plays a key role. Due to stimulation by foetally derived antigens, lymphocytes of healthy, pregnant women express progesterone receptors and in the presence of progesterone produce a 34-kDa mediator protein, named the progesterone-induced blocking factor (PIBF). PIBF induces a Th2 dominant cytokine production via inhibiting STAT4 and activating STAT6, inhibits maternal NK activity which results in decreased cell-mediated response thus exerts an anti-abortive effect. Though PIBF does not directly bind to IL-4 receptor-alpha, our aim was to determine the role of IL-4Ralpha in PIBF signalling. Furthermore, we investigated the effects of PIBF on the protein kinase C (PKC)/ Ca++ system which plays a key role in Th1/Th2 differentiation.
Materials and Methods: Confocal microscopy was used to detect the localization of IL-4Ralpha and PIBF receptor (PIBFR). To verify the involvement of IL-4-

Ralpha in PIBF signalling, IL-4Ralpha was silenced by oligonucleotides interfering with IL-4Ralpha mRNA. Assuming that the PIBF receptor is a GPI-anchored protein, PIBF-induced phosphorylation of STAT6 was tested in phosphatidylinositol-specific phospholipase C (PI-PLC) digested cells by Western blotting. The hypothesis that PIBF receptors float in glycosphyngolipid-cholesterol rafts was tested by depletion of cholesterol using methyl-β-cyclodextrin (MbCD). Proteins from PIBF-treated cells were reacted on Western blots with phospho-specific antibodies recognizing different PKC izoforms. Intracellular free calcium was measured by flow cytometry.
Results: Labelling of the IL-4Ralpha and simultaneous activation of the PIBF receptor with a FITC-labelled ligand revealed co-capping of the two binding sites. In IL-4Ralpha deficient cells the STAT6 activating effect of PIBF was markedly reduced. After PI-PLC treatment PIBF did not induce STAT6 phosphorylation while IL-4 retained the same effect. In MbCD treated cells neither PIBF, nor IL-4 were able to Tyr-phosphorylate STAT6, suggesting that not only the PIBFR but also the IL-4-Ralpha is enriched in lipid rafts. Both IL-4 and PIBF induced PKC phosphorylation which was abrogated by anti- IL-4Ralpha or anti-PIBF IgG pre-treatment. PIBF treatment did not alter intracellular Ca++-levels. Inhibition of PKCzeta or PKCtheta phosphorylation, but not that of PKCalpha/beta resulted in the loss of STAT6 and JAK1 phosphorylation by PIBF.
Conclusion: Our findings suggest the existence of a novel type of IL-4R, composed of the alpha-chain of IL-4R and the PIBFR. The PIBFR is a GPI-anchored protein that lacks cytoplasmic tail thus it uses the intracellular domain of IL-4Ralpha for signalling. Upon ligand-binding, the PIBFR enriched in lipid rafts forms a complex with the IL-4Ralpha subunit and activates the JAK1/STAT6 pathway. PIBF phosphorylates PKC via binding to the IL-4R, without affecting intracellular Ca++. Phosphorylation of PKCzeta and PKCtheta is required for JAK1 and STAT6 activation, whereas PKCalpha/beta is not involved. These findings explain the mechanism by which PIBF supports a Th2 dominant cytokine pattern.

Keywords: Progesterone, PIBF, PKC, STAT6, Th2

O-35: Macrophage Colony-Stimulating Factor Levels as a New Parameter to Predict Human IVF Outcome

Salmassi A[1]*, Mettler L[2], Walter Jonat W[3], Schmutzler A.G[4]

1. University Hospital, Dept. Gyn and Obst, Kiel, Germany
2. Reproductive Biological Laboratory, Kiel, Germany
Email: asalmassi@email.uni-kiel.de

Objective: M-CSF is a cytokine mediating the growth, proliferation and differentiation of various cell types including macrophages, trophoblast cells, and osteoclasts. It plays an important role in immunity reproduction, follicle development and ovulation. In the present study, we describe the important role of the changes in serum M-CSF levels during the menstrual cycle, in the process of follicular maturation, ovulation, implantation, pregnancy and their response to ovarian stimulation with recombinant (r) FSH
Materials and Methods: From an original sample of 95 IVF/ICSI patients (mean age = 33.8±5.4), serum and follicular fluid (FF) were collected on the day of follicular puncture (FP). The M-CSF levels were measured by ELISA technique. These patients were divided into two groups as follows: In Group 1 patients with the aetiology of tubal or male factor infertility were analysed for: a) correlation between serum and FF with respect to M-CSF and correlation between M-CSF and estradiol (E2) in serum; and b) comparison of M-CSF level in serum in response to ovarian stimulation and comparison of M-CSF level in serum between pregnant and non-pregnant patients. In Group 2: Patients (n=23) were monitored throughout the menstrual cycle until 4 weeks after embryo transfer. In this group, M-CSF levels in serum were analysed throughout the different ovarian cycle phases and gestation.
Results: M-CSF levels in FF were higher than in serum (p=0.01). M-CSF levels in serum increased from low, through moderate, to high response patients (p=0.001); pregnancy rates were 11.5%, 22.5% and 51.7%, respectively. M-CSF in serum increased throughout stimulation until the day of oocyte retrieval (p=0.006) and decreased until embryo transfer (ET, p=0.03). In the post-retrieval days, from the day of ET, through implantation, to the day of confirmation of pregnancy, the M-CSF levels of those patients who became pregnant (n=13) increased significantly (p=0.03) and reached their highest level. After implantation the M-CSF levels decreased slightly and reached a plateau during gestation.
Conclusion: our data show that M-CSF is involved in follicle development and ovulation and plays an important role in the maintenance of pregnancy. It could also be a predictor of embryo implantation for IVF outcome.

Keywords: Macrophage Colony-Stimulating Factor, Serum and Follicular Fluid, Response to Ovarian Stimulation, IVF Outcome

O-36: A New Treatment of Hirsutism with Myo-inositol. A Prospective Clinical Study

Unfer V[1]*, Minozzi M[2], Papaleo E[3]

1. AGUNCO, Rome, Italy
2. University of Rome, Rome, Italy
3. San Raffaele, Milan, Italy
Email: vunfer@gmail.com

Objective: The aim of this study was to evaluate the effects of myo-inositol on the lipid pattern and insulin sensitivity in hirsute women.
Materials and Methods: 129 hirsute women were enrolled to the first Institute of Obstetrics and Gynecology

(University La Sapienza, Rome, Italy) and evaluated at baseline and after receiving six months of myo-inositol therapy (4 gr/day). Body Mass Index, hirsutism score, serum levels of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, apolipoprotein B, lipoprotein(a), serum adrenal and ovarian androgens, fasting glucose and insulin concentrations were evaluated.
Results: The hirsutism score decreased after therapy (p value <0.001). Total androgens evaluated decreased, as a decrement of FSH and LH levels, while E2 levels increased. There was a slight decrement of total cholesterol levels but it isn't statistically important, an increment of HDL cholesterol levels and a decrement of LDL cholesterol levels. No significant changes were observed in serum triglyceride, apolipoprotein B and lipoprotein(a) concentrations. Fasting insulin levels and insulin resistance analyzed by homeostasis model assessment were reduced significantly after therapy.
Conclusion: Administration of oral myo-inositol significantly reduced the hirsutism score and hyperandrogenism and ameliorate the abnormal metabolic profile of women with hirsutism

Keywords: Myo-Inositol, Total Cholesterol, HDL Cholesterol, LDL Cholesterol, Triglycerides, Insulin Resistance

Genetics

O-37: Coexpression of Adrenomedullin and Its Receptors in the Reproducive System of the Rat: Effects on Steroid Secretion in Rat Ovary

Lei Li[1], Isabel Shi-Shan Hwang[1], Fai Tang[1, 3], Wai-sum O[2, 4]*, Yuk-YinLi[1]

1. Department of Physiology, University of Hong Kong, Hong Kong, China
2. Department of Anatomy, University of Hong Kong, Hong Kong, China
3. Centre of Heart, Brain and Healthy Aging, Hong Kong, China
4. Center of Reproduction, Development and Grwoth, Hong Kong, China
Email: owaisum@hkucc.hku.hk

Objective: The expression of adrenomedullin (ADM) in the reproductive system of the female rat and its effect on the secretion of estradiol and progesterone was studied.
Materials and Methods: In adult Sprague-Dawley rats, gene expression of ADM and its receptor components in the ovarian follicles, oviduct were analyzed by real-time PCR (qPCR). Ovarian follicles and corpora lutea isolated from stimulated immature rats were cultured to study the effects ADM on *in vitro* basal, FSH-stimulated estradiol secretion or eCG stimulated progesterone secretion.
Results: Ovarian ADM and Adm mRNA levels were decreased at estrus, whereas oviductal Adm mRNA levels were low at proestrus. Both tissues were shown to coexpress mRNAs encoding the calcitonin receptor-like receptor and receptor activity-modifying protein 1 (Ramp1), Ramp2, and Ramp3. Gel filtration chromatography of ovarian extracts showed two peaks, with the predominant one eluting at the position of authentic rat ADM(1-50) at estrus and at the position of ADM precursor at diestrus. Positive ADM immunostaining was localized in the granulosa and theca cells of the follicle and corpora lutea of the ovary. ADM inhibited FSH-induced estradiol secretion in 2-day-old follicles and also suppressed eCG-stimulated progesterone release in the corpora lutea was abolished by calcitonin gene-related peptide (8-37) and ADM (22-52), respectively.
Conclusion: The presence of ADM and the gene expression of ADM and its receptor components in the female reproductive system suggest a paracine effect of ADM on ovarian steroidogenesis.

Keywords: Estradiol, Follicle-Stimulating Hormone, Human Chorionic Gonadotrophin, Ovary, Progesterone

O-38: Reduction of Induced Transgenerational Genomic Instability in Gametes Using Vitamins E and C, Observed as Chromosomal Aneuploidy and Micronuclei in Preimplantation Embryos

Mozdarani H

Medical Genetics, Tarbiat Modares University, Tehran, Iran
Email: mozdarani_h@yahoo.com

Objective: Mutational events may be an indirect effect on genome stability which is transmitted through the germ line of chemically or physically exposed parents to their offspring. The consequences of germ cell mutations in subsequent generations include genetically determined phenotypic alterations without signs of illness, or reduction in fertility, or embryonic or prenatal death, more or less severe congenital malformations, or genetic diseases with various degrees of health impairment. In this study, the effect of induction of DNA damage during spermatogenesis cycle and preovulatory stage oocyte on the frequency of chromosomal abnormalities and micronuclei formation in preimplantation embryos generated by damaged sperm or oocyte after exposure to gamma rays in the presence or absence of vitamins E and C is investigated.
Materials and Methods: DNA damage was induced in male NMRI mice using gamma-rays, and then mated with non irradiated super-ovulated female mice in 6 successive weeks after irradiation in a weekly interval. In experiments involving irradiation of both male and female mice, irradiated male mice for 6 weeks post-irradiation were mated with female mice irradiated after induction of super-ovulation. To study the effects of vitamins E and C on the radiation induced DNA damage and consequently in the chromosomal abnormalities generated in preimplantation embryos, vitamin E at a concentration of 200 mg/kg and vitamin E at 100 mg/kg was administered to mice intera-

peritoneally 1 hour before exposing to radiation. Standard methods were used to prepare slides from pre-embryos for chromosome and micronuclei study.

Results: The rate of both aneuploidy and MN observed in embryos generated from irradiated male compared to control group dramatically increased ($p<0.01$). Frequency of aneuploidy and MN in embryos generated by mating both male and female irradiated mice was higher than that observed for those embryos generated by irradiated male mice alone. Cells at early spermatogenetic cycle were more sensitive to radiation and led to higher frequency of aneuploidy and micronuclei in preimplantation embryos. Exposure of male and female animals to gamma irradiation in the presence of vitamins E and C led to a considerable reduction in both chromosomal aneuploidy and micronuclei in generated preimplantation embryos by irradiated parents. Reducing factor for vitamin E was found about 2 and for vitamin C about 3. Also a direct correlation between aneuploidy and micronuclei formation was observed.

Conclusion: Results indicate that induction of DNA damage in gonads during spermatogenesis and pre-ovulatory stage oocytes may lead to unstable chromosomal aberrations and probably stable chromosomal abnormalities affecting pairing and disjunction of chromosomes in successive pre-implantation embryos expressed as aneuploidy and micronuclei. These types of chromosomal alterations may lead to impaired embryonic and fetal developments. Administration of vitamins E and C before irradiation effectively reduced the frequency of chromosomal abnormalities. The way these vitamins reduces genotoxic effects of radiation might be via radical scavenging mechanism or antioxidative effects. Higher dose reduction factor observed for embryos generated after vitamin C treatment might be due to water soluble nature of this vitamin or its direct involvement in DNA repair. This observation might have a great impact for cancer patients under radiotherapy, occupationally exposed individuals to physical and chemical DNA damage inducing agents and residents of high natural background radiation area who experience impaired gametogenesis and fertility.

Keywords: Spermatogenesis, Pre-Ovulatory Oocyte, Chromosomal Abnormalities, Pre-Implantation Embryo, Vitamins E and C

O-39: Infertility, Innate Immunity and Female Reproductive Tract

Reza Aflatoonian[1, 2]

1. Academic Unit of Reproductive and Developmental Medicine, The University of Sheffield, Level 4, Jessop Wing, Tree Root Walk, Sheffield, UK.
2. Departmenr of Endocrinology and Female Infertility, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: R.Aflatoonian@gmail.com

During the last decade the Toll Like Receptors (TLR) were found to be a major part of innate immune system. Several reports have demonstrated the existence of TLRs in different tissues and organs. However, little has been done to identify TLRs in the female reproductive tract (FRT). In addition little information existed regarding variation in TLRs in FRT during the menstrual cycle and also the influences of sex hormones on TLRs expression and function in this tract.

The distribution of TLR7-10 protein was detected by immunostaining in endometrium. Also RT-PCR was used to show the existence of TLR genes in human fallopian tube, endometrial tissue and OE-E6/E7 cells as a reliable model of fallopian tube epithelial cell. Q-PCR analysis was used to investigate relative expression of these genes during the menstrual cycle in human endometrium. In addition, separate and synergistic effects of sex hormones on TLRs were tested in OE-E6/E7 cells by using Q-PCR and ELISA. To confirm the specific effects of sex hormones in the study, antagonists against estradiol and progesterone were used.

The results showed that TLR7-10 proteins were present in endometrial epithelium and stroma. TLR1-10 genes were expressed in human fallopian tube and endometrial tissue. The mean relative expression of TLR genes was significantly higher during the secretory phase of the menstrual cycle in endometrium. In addition, it was demonstrated that TLR 1-6 genes and proteins were expressed in OE-E6/E7 cell line. Our data clearly showed that Estrogen has no effect on the expression of TLRs except TLR1 in OE-E6/E7 cells. In contrast, progesterone had an inhibitory effect on the expression of TLR1-4 genes in this cell line. Moreover, we proved that the production of IL-6 was significantly increased in the presence of TLR3 ligand (poly (I:C)), and both sex hormones had a suppressive and biphasic effect on the production of IL-6 in the presence and absence of poly (I:C). Our results also suggested that the estrogen receptor β and nuclear progesterone receptor B are likely to mediate the hormonal regulation of TLR3 as these two receptors are the two main estrogen and progesterone receptors in OE-E6/E7.

These results imply a potential variation in the expression and function of TLRs in FRT when different levels of sex hormones are present during different stages of the menstrual cycle. Clinically, Limited success in dealing with infertility issues and protection against sexually transmitted disease demonstrate the need for a greater understanding of the regulation of immune system in the female reproductive tract. Studying the function of TLRs in the female reproductive tract presents an exciting opportunity to further understand the regulation of innate immune system in the female reproductive tract. It seems sex hormones regulate the function and expression of TLRs in the female reproductive tract and therefore influence/regulate innate and adaptive immune function in this tract to protect against potential pathogens while providing an environment that supports an allogeneic foetus. How TLR function and expression is exactly regulated in reproductive tract by sex hormones would be a challenging but fruitful area of future research.

Reproductive Imaging

Ori-1: Intervention Management of Uterine Fibroma

Akhlaghpour A.A

Radiology Interventional Department, Noor Medical Imaging Center

Leiomyomata uteri are the most frequent myometrial disorders and the most common pelvic tumor in women. Although most women with uterine leiomyomas do not seek therapy, 20% to 40% of women in the reproductive age do have significant enough symptoms caused by these fibroids to cause the woman to seek and warrant therapy.
Since 1995 uterine fibroid embolization (UFE) or uterine artery embolization (UAE) was originally devised to reduce pelvic bleeding due to postpartum hemorrhage . it has been introduced as a treatment for symptomatic uterine myomas sparing the uterus.
Without a good blood supply, it has been shown fibroids will decrease in size between 30% and 50% and decrease in symptomatology.
Initial studies have shown that UAE can improve menorrhagia in 90% of patients at 1 year after therapy. On average, the volume of the fibroids decrease by 30%–60% and the associated symptoms (of mass effect) are successfully treated in 71% of patients.
Complications include postprocedure pain and postembolization syndrome possibly related to the release of cytokines and toxins from the ischemic tissue.
Vascular anastomotic communications between the uterine and ovarian arteries provide a route by which embolization materials can affect the ovarian blood supply and ovarian function, either permanently or temporarily. So Currently, UAE is not recommended as the first line of therapy in patients with infertility presumed to be caused by fibroids.
Magnetic resonance imaging–guided focused ultrasound surgery (MRIgFUS) is another groundbreaking minimally invasive alternative to surgery for fibroids.
Focused ultrasound causes local tissue thermal coagulation, ablates the target fibroid, and allows preservation of uterine function. It is a feasible and safe outpatient procedure that does not require hospitalization.
The procedure begins with the delivery of low-power (50–100 watt) sonication, with real-time thermometry acquired simultaneously. We will discuss these two techniques in detail and share our experience.

Ori-2: Embolization of Varicocele

Golestanha A

Shahed University, Tehran, Iran

Varicocele is the commonest curable cause of male infertility.
According to the statistical data collected from literature review there is
10-25% risk of recurrence after routine methods of varicocele operation.
If a surgeon is certain about accuracy of the surgical method, probable cause of recurrence would be presence of collaterals and anatomical variations, so embolization would be the method of choice in case of recurrence.

Ori-3: Breast Screening in Patient Undergoing ART Cycles

Salmati M

Department of Reproductive Imaging, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

Ori-4: First Trimester Screening for Chromosomal Anomaly

Tahmaspour A.R

The traditional method of screening for Down's syndrome has been maternal age where amniocentesis or chorionic villous sampling is offered to women aged 35 years or more. This results in the need for an invasive test in 15-20% of pregnant women with a detection of less than half of the fetuses with Donw's syndrome, because the majorly of affected fetuses come from the younger age group.
A more effective method of screening is based in the combination of:

- Maternal age
- A maternal blood sample for the measurement of the placental products of free
  ß-hCG and PAPP-A
- An ultrasound scan at 11-13 weeks:
  - to measure the collection of fluid behind the fetal neck (nuchal translucency)
  - to examine the fetal nose and palate
  - to measure the fetal heart rate
  - to assess the flow of blood across the tricuspid valve of the fetal heart and the
    ductus venosus

This new method of screening reduces dramatically the number of women requiring an invasive test from about 20% to less than 3% and at the same time increases the detection rate of Down's syndrome and other major chromosomal abnormalities form less than 50% to more than 95%

Poster Presentations

Andrology

P-1: Effects of Freezing and Thawing on Sperm Plasma Membrane Glycoconjugates: A Preliminary Study

Akyash F*, Talaei T, Esmaeilpour T, Bahmanpour S

Shiraz University, Shiraz, Iran
Email: akiash.n@gmail.com

Objective: Freeze and thaw have some detrimental effects on sperms. Molecular changes on the sperm surface can impact on fertility rate. We tested wheat germ agglutinin (WGA), peanut agglutinin (PNA) and Dolichos biflorus (DBA) to detect surface glycoconjugates before and after freezing
Materials and Methods: For this purpose, 45 healthy semen samples were frozen and thawed and sperm smears were prepared before and after freezing and thawing. The smears were stained with the lectins and also with acridin orange. The smears were studied by fluorescents microscopy and the intensities of the reactions to lectins were measured by image analyses software.
Results: The results indicated that the reactions of 46.67%, 34.09% and 73.34% of the specimens modified to PNA, WGA and DBA lectins respectively, after freezing and thawing. The cryopreservation caused both increase and decrease of the intensities of the reactions. It means that there are various mechanisms that impact on the carbohydrate contents of the sperm surface.
Conclusion: We conclude that cryopreservation affected the surface glycoconjugates at least in a subset of spermatozoa. These results might improve future application of sperm banking techniques.

Keywords: Sperm, Cryopreservation, Surface Glycoconjugates, Lectin

P-2: The Effects of Aminoguanidine on Epididymal Sperm Parameters in Varicocelized Rat

Alizadeh R[1]*, Abbasi M[1], Abolhasani F[1], Amidi F[1], Ragerdi Kashani I[1], Sobhani A. Gh[1], Pasbakhsh P[1], Hedayatpour A[1], Mahmoudi R[2]

1. Anatomy Department, Tehran University, Tehran, Iran
2. Anatomy Dpartmetn, Yasouj University, Yasouj, Iran
Email: nafiseh.alizadeh@google.com

Objective: Increased nitric oxide in the spermatic veins of men affected by varicocele has been reported. NO plays an important role in the varicocele-induced decrease of seminal quality. iNOS activity may play a role in the testicular dysfunction associated with varicocele during adolescence. iNOS activity was slightly increased in the leydig cells of varicocelized rats. It is a clinical case that inhibitors of NOS have succeeded in enhancing motility. Aminoguanidine is an inhibitor of iNOS. The objective of this study was to investigate the role of AG in efficiency of sperm parameters in varicocelized rat.
Materials and Methods: Twenty four wistar male rats divided into four groups. The group A and B underwent a left experimental varicocele. In group C (sham group), rats underwent a similar procedure but without any change on spermatic vein. Group D referred to as control. Animal in group A were killed 10 weeks after the operation. Epididymal sperm parameters were evaluated. Group B received 50 mg/kg AG intraperitoneally daily for ten weeks.
Results: Sperm parameters decreased significantly in the group A and the group B in comparison with group C and D ($p \leq 0.05$). The sperm parameters increased in the group B that received AG in comparison with the group A ($p \leq 0.01$). Group C (sham) did not show any significant alteration in sperm parameters in comparison with the control group.
Conclusion: These findings suggest that iNOS is an important mediator in the pathogenesis of varicocele. This study may support the concept that the use of iNOS inhibitor in infertile men with varicocele may be useful.

Keywords: iNOS Inhibitor, Sperm Parameters, Varicocele, Rat

P-3: Seminal Fluid & Hormonal Profiles among Iraqi Patients with Male Infertility

Al-Ahmer A.T.N.K.*, Mahmoud I.H., Al-Araji S.M.H.

College of Medicine, Babylon University, Babylon, Iraq
Email: aattnn88@gmail.com

Objective: The aims of this case-control study were to identify the seminal fluid patterns in Iraqi men with infertility as well as in fertile controls .Also to demonstrate the types of serum hormones (Follice Stimulating Hormone(FSH),Leutinizing(LH),Testosterone and Prolactin) abnormalities in the study groups
Materials and Methods: 81 Iraqi men with infertility and 30 fertile men who fulfilled the selection criteria to whom seminal fluid analysis (SFA) was performed according to WHO method.The patients group was subdivided by sperm concentration into azoospermic,olig oasthenozoospermic and oligozoospermic subgroups. Serum levels of the hormones (Testosterone,FSH,LH and Prolactin) were measured for patients and controls using ELIZA immunoassays.Seminal fluid analysis parameters mean levels and serum hormones levels were compared for the groups using Analysis of Variance test.
Results: Iraqi infertile men showed lower values for SFA parameters than did the controls.Patients with azoospermia showed the most remarkable hormonal

abnormalities especially in the levels of serum FSH and Testosterone. Patients also demonstrated multiple abnormalities in seminal fluid parameters. There was significant differences in the serum sex hormones levels between the patients and controls groups and among the infertile men subgroups.
Conclusion: Seminal fluid abnormalities among Iraqi patients with male infertility are multi-components.Hormonal profiles for these patients does not follow a single pattern.Patients with low sperm concentration and especially those with azoospermia are those that most likely will get benefit from hormonal assays.Serum FSH and Testosterone are the best 2 hormones for initial male infertility evaluation.

Keywords: Male Infertility, Seminal Fluid Analysis, FSH, LH, Testosterone, Prolactin

P-4: Histopathological Evaluation of Dopamine and Vitamin C Administration on Ischemia-Reperfusion Injury after Testicular Torsion in Rats

Azizollahi S*, Babaei H, Derakhshanfar A

Veterinary Medicine, Shahid Bahonar University, Kerman, Iran
Email: saeedazizollahi@yahoo.com

Objective: Testicular torsion is the twisting of the spermatic cord, which cuts off the blood supply to the testicle. The main pathophysiology of testicular torsion is ischemia-reperfusion injury caused by the twisted spermatic cord and its release. Reperfusion component typically involves the generation of the reactive oxygen species with the return of blood flow after the ischemia. The aim of the present study was to investigate the effects of dopamine, a vasodilator, and vitamin C, an antioxidant, on ischemia reperfusion injury due to testicular torsion-detorsion (T/D).
Materials and Methods: Thirty adult male NMRI rats were divided randomly into six groups each containing 5 rats. Testicular ischemia was achieved by twisting the left testis 720° clockwise for 4 hour, and then reperfusion by detorsion. Group 1 was for the basal values. Group 2 was designed as sham-operated without any T/D. Group 3 had only T/D. Group 4 received vitamin C (100 mg/kg, i.p) just after detorsion. Group 5 received dopamine (0.01 mg/kg, i.p) after 4 hours T/D and group 6 received combination of vitamin C and dopamine. The animals in each group underwent orchiectomy 48 hours after detorsion and the testicular tissues were fixed in 10% formaldehyde for histopathological examinations.
Results: In groups 1 and 2, microscopic features were normal. In group 3, all of the seminiferous tubules were damaged. In some tubules only the basement membrane was seen. Pyknosis of spermatocytes nuclei was prominent. Germ cells and sertoli cells were decreased and underwent degeneration and necrosis. Interestingly in group 4, only a few degenerative changes (6.8%) were observed in tubules. In group 5, approximately 90% of tubules were underwent fully or partial degeneration and in group 6 only few normal tubules were found (2.2%).
Conclusion: Vitamin C administration exerts a beneficial effect on testicular ischemia-reperfusion injury but not dopamine or even dopamine and vitamin C combination.

Keywords: Dopamine, Vitamin C, Testicular Torsion, Ischemial-Reperfusion

P-5: A Study of the Contraceptive Activity of a New Derivative of Nifedipine in Male Adult Rats

Bahmanzadeh M[2], Hassanein P[1]*

1. Bu-Ali Sina University, Hamedan University, Hamedan, Iran
2. University of Medical Scieces, Hamedan, Iran, Hamedan, Iran
Email: bahmanzadeh12@yahoo.com

Objective: 1,4Dihydro 2,6Dimethyl 4(4.Nitrophenyl) 3,5Pyridinedicarboxylic acid Dimethyl ester (DDNPD) is a new analogous of Nifedipine that has no effect on calcium channels of cardiovascular system. Because Nifedipine can reduce fertility in males (through effect on calcium channels on sperm membrane and inhibition of acrosomal reaction), this study was done to evaluate the effect of DDNPD on reproductive system physiology and fertility in male rats.
Materials and Methods: In this research, DDNPD was injected 10mg/kg twice a day subcutaneously to the test group of adult male rats for 50 days. Control group treated with propylene glycol 1 mg/kg twice a day in the same way.
Results: In the assessment of some fertility indices, we saw a significant decrease in motility and viability rate of sperm ($p<0.001$), the epididymal sperm reserve rate ($p<0.001$) and fertility rate ($p<0.001$) in the test group but there was no difference in testosterone level between the groups.
Conclusion: These results are conclusive of post testicular antifertility effect of DDNPD if injected 10 mg/kg for 50 days to male adult rats.

Keywords: Calcium Channels, Infertility, Rats, Spermatozoid

P-6: Developmental Effects of Lead Acetate on Seminiferous Tubules in Wistar Rats

Dorostghoal M*, Moazedi, Sorooshnia F

Biology Department, Shahid Chamran University, Ahvaz, Iran
Email: mdorostqoal@yahoo.com

Objective: In recent years concerns have been raised about the decrease of male infertility from exposure to environmental pollution such as heavy metals. So, developmental effects of lead acetate were studied on

seminiferous tubules structure during postnatal development in male Wistar rats.
Materials and Methods: The male pups randomly divided in control and experiment groups. The mothers of experiment groups received 100, 200 and 300mg/L/day lead acetate via drinking water during lactation. The testes of pups removed their weights were recorded and fixed in Bouin's solution. Then, the volumes of testes were measured by Cavalieri method. For microscopic studies, following tissue processing, 5µm sections were stained with haematoxylin-eosin.
Results: Macroscopic results showed that the means of testis weight of experiment groups increase during early and decrease at the late of postnatal development in comparison with control group. Microscopic results indicated that decreasing of germinal epithelium height and presence of vacuoles in germinal epithelium comparison with control group. Also, the means of testis volume, relative volumes of seminiferous tubules and germinal epithelium significantly decreased in experiment groups during all stage of postnatal development ($p<0.05$). Moreover, the means of seminiferous diameter and epithelium height significantly decreased in experiment groups during all stages of postnatal development ($p<0.05$).
Conclusion: Present study indicate that lead acetate could affect postnatal development of seminiferous tubules in rats and decrease spermatogenic activity of adult Wistar rats that have been exposured to it during neonatal period.

Keywords: Male Infertility, Seminiferous Tubules, Postnatal Development, Lead Acetate

P-7: Stereological Effects of Di (2-Ethylhexyl) Phthalate on Postnatal Development of Testis in Wistar Rat

M. Dorostghoal*, Moazedi, F. Sorooshnia

Biology, Shahid Chamran University, Ahvaz, Iran
Email: mdorostqoal@yahoo.com

Objective: Phthalate esters extensively used as plasticizers in the plastic industry, have been shown to cause reproductive toxicity in both developing and adult animals. So, present study was aimed to determine quantitative effects of di-(2-ethylhexyl)-phthalate (DEHP) on postnatal development of rat testis.
Materials and Methods: For this purpose, pregnant females rat were orally exposed to various doses (0, 100, and 500 mg/kg per day) of DEHP during gestation and lactation. Testes of offspring were removed at 7, 14, 28, 60 and 90 days after birth, their weight recorded and fixed in bouin's solution. Then, the volumes of testes were measured by Cavalieri method. For microscopic studies, following tissue processing, 5 µm sections were stained with haematoxylin-eosin.
Results: The results were showed that the means of testis weight decreased significantly ($p<0.05$) in low dose group at 90 days after birth and in high dose group from 28 days after birth in compared with control group. Total volume of testis at 90 days after birth in both low and high dose groups reduced significantly ($p<0.05$) 14.54 and 10.03 percent in compared with control group, respectively. Relative volume of seminiferous tubules showed significant reduction from 14 days after birth in both experiment groups. Seminiferous tubules diameter at 90 days after birth decreased days significantly ($p<0.05$) 15.20 and 21.57 percent in compared with control group, respectively.
Conclusion: Present study determine degree of quantitative variations in testicular parameters during postnatal development in Wistar rats exposed to di-(2-ethylhexyl)-phthalate (DEHP).

Keywords: Di-(2-Ethylhexyl)-Phthalate, Postnatal Development, Testis, Stereology

P-8: Effect of L-carnitine Supplement on Semen Parameters in Men with Idiopathic Infertility

Eidi M[1], Pouyan O[2], Shahmohammadi P[3*], Eidi A[3]

1. Biology Department, Islamic azad University, Varamin, Iran
2. IVF, Shariati Hospital, Tehran, Iran
3. Biology Department, Islamic Azad University, Tehran, Iran
Email: maryameidi@gmail.com

Objective: The benefit effect of L-carnitine is proposed for treatment of various dysfunctions of sperm in infertile men. So, in the present study, effect of L-carnitine supplement on sperm parameters in men with idiopathic infertility is evaluated.
Materials and Methods: Thirty infertile men, ages 20 to 40 years, with the following baseline sperm selection criteria (sperm count $<66.6 \times 106$, motility $< 30\%$, viability $<60\%$, normal morphology $<35\%$) completed this study. Patients underwent L-carnitine therapy 3 g/day; the study design was 3 and 6 months of therapy. Semen analysis was performed according to World Health Organization guidelines. Sperm parameters included liquefaction, pH, volume, sperm count, motility, viability and normal morphology.
Results: The results showed that L-carnitine supplementation increased sperm count, motility, viability and normal morphogy and pregnancy rate after 3 months ($p<0.01$) and 6 months ($p<0.001$), significantly. Also, L-carnitine supplementation increased sperm motility and viability in idiophatic infertile men after 3 and 6 months ($p<0.001$). On the other hand, 5 couples became pregnant during the study.
Conclusion: The present study indicated that L-carnitine supplementation is an appropriate drug for treatment men with idiopathic infertility.

Keywords: L-Carnitine, Sperm Parameters, Idiopathic

Infertility, Men

P-9: The Effect of Various Doses of Nicotine on Spermatogenesis Maturation and Sperm Parameters in Adult Mice

Fallah M*, Ghasemi F.M, Ranjbar S, Jalalsafari M

Histotechnique lab, Anatomy Department, GUMS, Rasht, Iran

Objective: Cigarette smoking is associated with impairment of testicular function. Nicotine is a major component of cigarette smoke. In the present study we aimed to demonstrate the effect of various doses of nicotine on sperm parameters and quality of spermatogenesis in adult mice.
Materials and Methods: Male adult NMRI mice were divided into four groups. 1) control group were received normal salin. The animals in experimental groups of 2, 3 and 4, were received nicotine at the doses of 0.1, 0.2 and 0.4 mg/100 g. BW respectively. Both nicotine and vehicle were administered intraperitoneally for 21 days. Evaluations were made by determining Johnson's score, epididymal sperm content, motility and morphology, leydig cells volume and serum concentration of testosterone. Statistical analysis was performed by ANOVA test.
Results: Nicotine caused a significant reduction in quality of spermatogenesis, sperm content , leydig cell volume and serum concentration of testosterone in groups of 3 and 4($p<0.05$). Administration of nicotine in dose of 0.1 mg/100g. BW did not affect on the quality of spermatogenesis and sperm content. However both sperm morphology and sperm motility ($p< 0.05$) and testosterone level ($p<0.001$) were decreased significantly.
Conclusion: These findings suggest that administration of nicotine for 21 days leads to a deficiency in sperm characteristics, maturation of spermatogenesis and secretory dysfunction of the leydig cells. The adverse effects of nicotine is increased in a dose dependent man\ ner.

Keywords: Nicotine, Spermatogenesis Maturation, Sperm Parameters

P-10: Survey the Frequency of Varicoceles in Men that are Referred to the Fatemeh Zahra Infertility Center in 1387

Firoozpour M*, Esmaeilzadeh S, Yosofnia Pasha Y.R, Asefnejad F

Fatemehzahr Infertility Research Center, Babol, Iran
Email: sesmael@yahoo.com

Objective: survey the Varicoceles's rate and intensity in patients who are referred To the Fatemeh Zahra(s) Infertility center.
Materials and Methods: In this field of descriptive study, the 713 file's information of infertile couple that referred to the Fatemeh Zahra(s) infertility center in 1387 are surveyed. The information which related to fertility's action is registered. The medical examination of Varicoceles were done by urologist with physical medical examination way, and at least results were surveyed by chi-square test
Results: The frequencies of the first and second infertility were in order 73/5% and 26/5%.The average of men's age in group with first infertility 9/72 years. ± 6/18 years and in group whit second infertility 34/69 ± 30/2 Varicoceles frequencies was totally 34% and in men with the first and second infertility were ordered 35%and 32% that didn't show meaningful difference. The most frequency of Varicoceles intensity, grade II by 57% and left Varicoceles by 67%. The most percent of Varicoceles is in under 40-year-old group with 34%.
Conclusion: with surveying the frequency of Varicoceles in this center. We can understand the rage of infertility patients in area .and evaluate according to age, kind of infertility and intensity of it to provide next detective and curative couple's programs, even to prevent before of the marriage.

Keywords: Varicoceles, Male Infertility, First Infertility, Second Infertility

P-11: Testosterone Enanthate Suppresses Apoptosis in Male Germ Cells Induced by Busulfan

Ghasemi F.M[1]*, Faghani M[2], Nasiri E[2], Bahadori M.H[2], Soltani B[1]

1. Cellular and Molecular RC, Guilan university, Rasht, Iran
2. Histotecnique lab,Guilan university, Rasht, Rasht
Email: parsahistolab@gmail.com

Objective: This study was performed to investigate the suppressive effect of testosterone enantate on male germ cells apoptosis induced by busulfan in rats.
Materials and Methods:
Sprague- Dawley rats were divided into 4 groups: 1. a control group given cotton seed oil, 2. a group given two doses of busulfan 10 mg/kg intraperitoneally, 21 days apart, 3. a group given two doses of testosterone enantate 8mg/kg subcutaneously, 21 days apart, 4. a group given both testosterone anantate and busulfan. Evaluations were made by determining johnsen's score and apoptosis were assayed by terminal- deoxynucleotidyl- transferase-mediated dutp nick end labeling (TUNEL).
Results: Recovery status and johnsen's score in group 4(busulfan + testosterone) were significantly higher than group 2(busulfan) 7.4 ± 0.20 VS 4.1 ± 0.22, TUNEL positive cells were significantly more numerous in busulfan treated group than control. While testosterone significantly attenuated morphological changes and germ cell apoptosis in testis damaged by busulfan.
Conclusion: These results suggest testosterone enantate have chemoprotective effect on male germ cells against busulfan-induced toxicity partly by decreasing of apoptosis.

Keywords: Chemotherapy, Testosterone Enanthate, Germ Cell Apoptosis, Testis

P-12: Male Infertility and Glutathione

Hosseini S*

Shahid Beheshti, Tehran Nursing and Midwifery, Tehran, Iran
Email: saeedeh38@gmail.com

Objective: "Glutathione , is a tripeptide" and known as GSH , just might be one of the most extraordinart overall health - boosters of modern nutritional medicine. GSH may help with everything from heightened immune system functioning to overcoming addictions to anti-aging and objective ." Many people are aware that sperm counts have dropped by half in the last 50 years, and that modern men have 20 percent less semen volume than their fathers. Persistent organic pollutants (POPs) and endocrine-disrupting chemicals from normal , everyday plastics are known to cause reproductive damage , Damage tosperm caused by exposure to common chemicals like alcohol and pesticides in food has been linked to lowered intelligence and behavioral disorders in children . Studies show that anti-oxidant supplementation - glutathione in particular - can improve sperm quality, and possibly increase your chances of conceiving .
Materials and Methods: This is review article.
Results: Mammalian spermatozoa are coated by a membrane rich in polyunsaturated fatty acids . These fatty acids are extremely susceptible to oxidative damage by free radicals or Reactive Oxygen Species (ROS) by a process called lipid peroxidation (LPO) . Lipid peroxidation damages the sperm cell membrance . It is considered to be the key mechanism of ROS- induced sperm damage and leads to * Loss of sperm motility * Abnormal sperm morphlogy * Reduced capacity for oocyte penetration * Infertility To protect sperm from damage, the body depends on powerful antioxidant enzymes in the body such as superoxide dismutase (SOD), catalase, and glutathione/reductase (GPX/GRD). Some amount of all the antioxidant enzymes , which may protect spermatozoa from axidative attack, are also made by the epididymis during storage. The glutathione peroxidase/reductase enzymes play a central role in the defense against oxidative damage in human sperm.
Results and Discussion: A decrease in levels of reduced glutathione (GSH) during sperm production is known to disrupt the membrane integrity of spermatozoa due increased oxidative stress . Intracellular glutathione levels of spermatozoa are known to be decreased in certain populations of infertile men .
Conclusion: Glutathione is not only vital to sperm antioxidant defenses , but selenium and glutathione are essential to the formation of " phosholipid hydroperoxide glutathione peroxidase" -an enzyme present in spermatids - which becomes a structural protein in the midpiece of mature spermatozoa. When either substance is deficient, it can lead to instability of the mid-piece of the spermatozoa, resulting in defective motility. Free radical scavengers - such as glutathione-that restore the structure and function of poly - unsaturated fatty acids (PUFA) in the cell membrane, can be used to treat these cases.

Keywords: Glutathione, Spermatozaoa, Infertility

P-13: Celecoxib and Male Infertility - An Experimental Design

Jahanpour N[1]*, Jahanpour F[2], Azodi P[3]

1. Physiology Department, Ministry of Education, Shiraz, Iran
2. Nursing Faculty, Bushehr University, Bushehr, Iran
3. Paramedical Faculty, Bushehr University, Bushehr, Iran
Email: f_jahanpour@yahoo.com

Objective: Celecoxib is a nonstroidal anti-inflammatory drug .This is a selectiveCox-2 inhibitor.Nowadays this drug uses as an analgesic,antipyretic and anti-inflammatory agent frequently. In some patients that used celecoxib for a long time, unusual effect of this drug may be seen. The goal of this survey is assess the effect of this drug on male-reproductive system functions
Materials and Methods: This survey done for study of effect of celecoxib on rat reproductive system , specially on spermatogenasis and the level of blood testosterone hormone. In this manner histologic studies and measuring of weight (testis, prostate, seminal vesicle and epidydimis) and the level of blood testestron are done. 50 rat with 200-230 gr. weight selected and compared in 5 groups.control group (no drug given), sham group (solvent drug: Di- methyl sulfoxide), 3 cases group (orally celecoxib 10, 20 and 40 mg/kg given daily) for 15 days. In the end of 15 days heart blood sampling for measuring serum testestron level accomplished after that reproductive systems separated and prepared for histologic study.
Results: Result showed no significant differences in mean weight of body testis, epidydimis and seminal vesicle in control and case groups. But significant differences are seen in the mean weight of prostate per body weight in case group (40 mg/kg) in compared with control group. That is due to antimalignant effect of celecoxib on prostate. No differences seem between control and case groups in arrangement mode, nuclei shape and cytoplasm in histologic examination in spermatogonia and primary spermatocytes in transverse section of seminiferous tubules celecoxib in case group (40 mg/kg) can decrease lydige cells number, that is due to inhibited prostaglandin synthesise, for the result of cox-2 inhibitor and decreased level of testosterone hormone.
Conclusion: It looks that number of sertoli cells in control and case groups are differences. So that in case group (40 mg/kg) number of sertoli cell's decreased due to decrease testestron level. This can cause production of abnormal sperms. In the survey can conclude that use of high doses of celecoxib can decreased size

and number of lydig cells and this is cause of decreased testosterone hormone.

Keywords: Celecoxib, Mail, Infertility, Testosterone Hormone

P-14: Yoga Therapy for Male Subfertility

Sinsen Joseph*, Logesh Hegde

City Point Medical Center, Dubai, UAE
Email: drsinsen@gmail.com

Objective: To augment male isubfertility treatment by using Yoga therapy as an adjuvant therapy.
Materials and Methods: Patients from city point medical center.
Results: Yoga therapy along with homeopathy tratment enhances spermatogenesis.
Conclusion: Yoga therapy can be considered as an adjuvant therapy for male subinfertlity.

Keywords: Yoga, Male Subinfertility, Asanas, Pranayama

P-15: Evaluation of Androgenic Activity of Allium Cepa on Spermatogenesis in Rat

Khaki A[1]*, Fathiazad F[2], Nouri M[3], Hamadeh M[4], Khaki A.A[5]*, Ghafari-Novin M[6]

1. Department of Veterinary Pathology (YRC), Islamic Azad University Tabriz Branch, Tabriz, Iran
2. Department of Pharmacognosy, Tabriz University of Medical Sciences, Tabriz, Iran
3. Department of Biochemistry Science, Tabriz University of Medical Sciences, Tabriz, Iran
4. Department of Obstetrics and Gynecology, University of Saarland, Humborg, Germany
5. Department of Anatomical Science,Tabriz University of Medical Sciences, Tabriz, Iran
6. Department of Cell & Molecular Biology Research Center, Shahid Beheshti University M.C, Tehran, Iran
Email: arashkhaki@yahoo.com

Objective: Allium cepa (onion) has a good effect on diseases treatment worldwide and have been used since ancient times as a medicinal and food origin. Recently several reports have shown that onion has high antioxidant activity. As antioxidants have essential effect on sperm health parameters, we investigated the effect of onion bulbs fresh juice in Spermatogenesis cycle in rats.
Materials and Methods: Wistar male rat (n=30) were allocated into three groups, control (n=10) and two test groups (each of ten). Animals in test groups were subdivided into groups of 2 that received fresh onion juice equivalent to 0.5&1g/Rat/day fresh onion. Fresh onion juice was administered with gavages for 20 consecutive days. Animals were kept in standard condition. On twentieth day, the testes of rats in the all groups were removed and sperm was collected from epididymis and was prepared for analysis.
Results: Serum total testosterone significantly increased in whole test groups ($p<0.05$) and level of LH significantly increased only in the group that received the high dose of fresh onion juice, ($p<0.05$), but the level of FSH did not differ between experimental and control groups. The percentage of sperm viability and motility in both test groups significantly increased ($p<0.05$), but the sperm concentration significantly increased only in the group that received the high dose of freshly extracted onion juice, ($p<0.05$). It was evident that there was no difference on sperm morphology and testis weight in test groups comparing to control group.
Conclusion: In our study freshly prepared onion juice has significantly affected the sperm number and percentage of viability and motility; it seems that using 4g/kg of freshly prepared onion juice is effective in sperm health parameters.

Keywords: Allium Cepa, Infertility, Spermatogenesis

P-16: Fertility and Spermatogenic Outcome of Varicocelectomy in Ouroperated Infertile Male Cases; A Prospective Study

Moslemi M.K*, Hashemeiieh S

Urology, Kamkar Hospital, Qom Medical Sciences University, School of Medicine, Qom, Iran
Email: moslemi_urologist@yahoo.com

Objective: This is a prospective 2 year study that we evaluated outcome of varicocelectomy from the point of semen indices improvement and fertility outcome.
Materials and Methods: From the beginning of 2006 to the end of 2008 we operated 123 infertile male with varicocel .21 cases were excluded from the study .in the remaining 102 cases,95 left varicocelectomy performed,5 right varicocelectomy and 2 bilateral varicocelectomy performed.age ranges was 18 to 38 years.range of infertility was 1 -12 years.infertility duration in 60% was 2-3 years,in 10% one year and in 30% was more than 3 years.40% has grade 1 or subclinicalvaricocel,35% G 2 and 25% G 3.all of the cases operated by inguinal incision,with naked eye.
Results: In general 25% of cases conceived normal pregnancy.semen analysis indices improved in 68% of cases.no improvement in S/A is noted in28%,and we had severity in indices of 7%.this occurred predominantly in cases with primary testicular failure or increased FSH level.or sperm count lower than 1 million /ml.
Conclusion: Varicocelectomy is a safe,easy and cheap procedure that if done by skilled urologist can lead to excellent outcomes ,that is highly comparable with ART methods from every point of view ,especially for acheving normal conception.

Keywords: Varicocel

P-17: Contraception Methods in Iranian Traditional Medicine and Ruta Graveolens L. Effects on

Human Spermatozoid

Naghibi Harat Z[1]*, Ebadiani M[2], Sadeghipour H.R[3], Sadeghi M.R[4], Kanalinejad M[5], Eshraghian M.R[6]

1. Arash Hospital, Tehran Medical University, Tehran, Iran
2. Traditional Medicine, Tehran Medical University, Tehran, Iran
3. Physiology, Tehran Medical University, Tehran, Iran
4. Endocrinology and Embryology, Avesina Institute, Tehran, Iran
5. Pharmacognosy, Shahid Beheshti University, Tehran, Iran
6. Biostatics, Tehran Medical University, Tehran, Iran
Email: drsinsen@gmail.com

Objective: Contraception had been a matter of concept in modern medicine from the sixties when World Health Organization asked governments to make coalitions with to search and find safe and reliable contraception methods. But in Iranian Traditional Medicine (TIM), contraception had been used more than thousand years ago. The ancients know about different contraception methods both for men and women, for short time or lifelong. They believed that contraception methods should be kept as a secret and it could be used only in special situation; when pregnancy was a danger for woman.
Materials and Methods: Here we do a search in the most important TIM manuscripts and classified the different contraception methods as following. Besides we examined the effect of decoction, boiled and different fractions of Ruta graveolens L. on human sperm characteristics *in vitro*.
Results: According to our findings, usage of this plant as a male contraceptive is unique to TIM. The results showed that their choice was a right one because coumarins of this plant in addition to its hyperosmolarity and acidic pH could immediately immobilize the spermatozoa and arrest the acrosomal reaction. Meanwhile there was no adverse effect on viability, mitochondrial function or chromatin structure. Classification of contraception methods in TIM: 1- Changing intercourse position in order to diminish the semen arrival to female genitalia. 2- Rapid removement of semen from female genitalia. 3- Mechanical barriers 4- Vaginal suppositories with different materials 5- Topical application of remedies on penis called "Tela" 6- Oral contraceptive for both men and women 7- Usage of "Talyghat"
Conclusion: The results showed that TIM used a wide range of methods for contraception which some were so ingenious. The experimental findings support the TIM unique idea about usage of Ruta graveolens L. as contraception and it reveals that knowing more about traditional remedies and performing more trials, may let to find new ways for contraception in future.

Keywords: Iranian Traditional Medicine, Contraception, Human Sperm

P-18: Effect of Growth Hormone on Testicular Dysfunction Induced by Methotrexate (MTX) in Rats

Serati Nouri H[1]*, Azarmi Y[2], Movahedin M[3]

1. Veterinary Medicine, Islamic Azad University, Tabriz, Iran
2. Pharmacology, Tabriz Medical University, Tabriz, Iran
3. Anatomical Sciences, Tarbiat Modarres University, Tehran, Iran
Email: hamed.serati@gmail.com

Objective: Methotrexate (MTX) is a chemotherapeutic agent causing defective oogenesis and spermatogenesis. This study was planned to see the role of human growth hormone (GH) on testis recovery after treatment with methotrexate.
Materials and Methods: The forty male Wistar rat were selected and randomly divided into four groups (n=10); control (vehicle), GH group (0.3mg/kg GH for 28 day, IP), MTX Group (MTX 1mg/kg /week for four week, IP) and GH/MTX group (0.3mg/kg GH for 28 day plus 1mg/kg /week MTX for four week, IP)). During day 14 and 28, five rats from each group were sacrificed and the testes of rats in all groups were removed and sperm was collected from epididymis and then prepared for analysis.
Results: MTX causes significant increase in interstitial tissue and capsular thickness and decrease of testicular and body weight ($p<0.05$). Also it causes significant decline in seminiferous tubules diameter and epithelium thickness ($p<0.05$). There were no obvious changes seen in morphmetrical parameters between MTX/GH and control groups. In MTX group, sperm parameters decreased significantly ($p<0.05$). Administration of GH plus methotrexate reduced its effects on sperm parameters and testosterone concentration.
Conclusion: These results suggested that GH had a protective effect on approximately all destructive effects caused by MTX in rat testes and improved sperm parameters.

Keywords: GH, MTX, Spermatogenesis, Testis, Rat

P-19: Evaluation of Fas Positive Sperm and Complement Mediated Lysis in Subfertile Individuals M.H.

Soleimani M[1]*, Nasr Esfahani MH[2], Tavalaee M[2], Aboutorabi R[1], Adib M[1], Janzamin M[3], Kiani A[2]

1. Isfahan University, Isfahan, Iran
2. Department of Reproduction and Development, Royan Institute for Animal Biotechnology, ACECR, Isfahan, Iran
3. Department of Regenerative Medicine, Royan Institute for Stem Cells Biology and Technology, ACECR, Tehran, Iran
Email: soleimanianmaryam@yahoo.com

Objective: Apoptosis appears to have an essential role in the control of testis germ cell number and Fas-mediated apoptotic pathway has been implicated in controlling apoptosis during spermatogenesis in a number of mammalian species .The apoptotic marker, Fas ,was detected in ejaculated sperm, with a higher incidence of Fas positivity in infertile men. In this study each sample

was analyzed for expression of Fas by Flowcytometry. Sperm shows Fas positive but PI negative (early apoptotic), Lysis was activated by antibody-antigen complexes via Fas receptor and Complement.
Materials and Methods: Semen samples were obtained from 73 patients referring to Isfahan Fertility and Infertility Center . To Complement mediated lysis each samples treated with 5µl Purified Mouse Anti-Human CD95 mAb and then the cells incubated for 1 hour at 37 ºC with 25 µl Rabbit complement To demonstrate the effect of complement-activating, and the degree of cell lysis, PI was used.
Results: Fas expressions of sperm in subfertile individuals were much lower and with a mean of 4.1%. No significant difference in percentage PI was detected before and after activation of classical pathway ($p = 0.53$).
Conclusion: No significant differences in percentage of PI, was detected before and after activation of complement classic pathway. Therefore, this may conclude that Fas system or possibly abortive apoptosis is likely none functional.

Keywords: Apoptosis, Flowcytometry, Infertility, Fas, Complement, Lysis

Embryology

P-20: Protective Effects of Ionic and Non-Ionic Media on the Najdi Bull Sperm During Cool Storage

Afrough M[1]*, Barati F[1], Papahn A.A[2]

1. Clinical Scieces Departemtn, Shahid Chamran University, Ahvaz, Iran
2. Basic Scieces Department, Shahid Chamran University, Ahvaz, Iran
3. Allam-Al-Hoda Research Center, Baseej Deneshjoie, Ahvaz, Iran
Email: baratif@scu.ac.ir

Objective: Cool storage is a way to preserve sperm for short period of time in Assisted Reproductive Technologies (ART). The aim of this study was to find effects of two iso-osmotic ionic (Tyrods solution) and non-ionic (Whole cow milk) in the context of two environment (25 and 5ºC) and two incubation time (15 and 60 min).
Materials and Methods: The Semens collected from five Najdi bulls in the station for supporting Najdi cow cattle and transported to the lab in 37ºC light protected tubes. After primary sperm analysis, the semens were equally splited into two main aliquots according to the media (Tyrods or Milk). The aliquots suspended with two equal volumes of media and the final suspension divide into two aliquots equally for incubation in 25 or 5ºC. The suspensions incubated and sperm analysis undertook after 15 and 60 minutes of incubation. Sperm viability assessed using Eosin/nigosin and its progressive motility were done with conversional procedures. In this factorial design effects of media, incubation temperature and incubation time were analyzed using GLM procedure of SAS. Data expressed as Lsmeans±ESM.
Results: Sperm viability decreased at 5ºC compare to 25ºC after 15 min of incubation within both media ($p<0.05$). Milk protected the sperm viability better than Tyrods after 60 min of incubation in 5ºC ($p<0.05$). In spite of good preservability of sperm viability in Tyrods at 25ºC ($p>0.05$), sperm viability decreased after 60 min of incubation in Milk at this temperature compare to 15 min of incubation ($p<0.05$). There was no difference between 15 min incubation of both media at 25ºC ($p>0.05$). Sperm motility decreased severely at 5ºC compare to 25ºC ($p<0.05$). However, Milk cause to severe decrease ($p<0.05$) the sperm motility after 60 min compare to Tyrods.
Conclusion: Cool storage of dilute semen is good way to protect the sperm parameters for short period of time. Ionic and non-ionic solutions are efficient in protecting the sperm parameters in this way.

Keywords: Sperm, Cool Storage, Bull, Tyrods

P-21: Effects of Subfertility Cause, Smoking and Body Weight on the Success Rate of IVF

Akhbardeh M

Washington University WSPA, Washington, USA
mahdi_akhbardeh14@yahoo.com

Objective: We investigated the separate and combined effects of smoking and body mass index (BMI) on the success rate of IVF for couples with different causes of subfertility.
Materials and Methods: The success rate of IVF was examined in 8457 women. Detailed information on reproduction and lifestyle factors was combined with medical record data on IVF treatment. All IVF clinics in The Netherlands participated in this study. The main outcome measures were live birth rate per first cycle of IVF differentiated for the major predictive factors.
Results: For male subfertility the delivery rate per cycle was significantly lower than unexplained subfertility, OR of 0.70 (95% CI 0.57–0.86); for tubal pathology, the delivery rate was slightly lower, OR = 0.86 (95% CI 0.70–1.01). Smoking was associated with a significantly lower delivery rate was slightly lower; for OR = 0.72 (95% CI 0.61–0.84) and a significantly higher abortion rate compared to non-smoking delivery rates of 21.4% and 16.4%, respectively (p=0.02). Women with a BMI of 27 kg/m2 had a significantly lower delivery rate, with an OR of 0.67 (95% CI 0.48–0.94), compared with normal weight women (BMI 20 and <27 kg/m^2).
Conclusion: Both smoking and overweight unfavourably affect the live birth rate after IVF. The devastating impact of smoking on the live birth rate in IVF treatment is comparable with an increase in female age of >10 years from age 20 to 30 years. Subfertile couples may improve the outcome of IVF treatment by lifestyle changes

Keywords: Smoking, Subfertility, Body Weight

P-22: Effects of Alcohol and Nicotine Co-Administration on Rat Sperm Parameters

Saeid Azizollahi[1]*, Arash Saveazad[2], Seyed Noureddin Nematollahi-Mahani[2], Masoud Ezzatabadipour[2], Zohreh Mirkahnooj[2]

1. Department of Veterinary Shahid Bahonar University, Kerman, Iran
2. Department of Anatomy, Kerman University of Medical Sciences, Kerman, Iran
Email: saeedazizollahi@yahoo.com

Objective: Toxic effect of alcohol and nicotine on the other organs of body has been proven to some extent. Effects of oral alcohol and subcutaneous nicotine co-administration on rat sperm parameters are the aim of this study.
Materials and Methods: Thirty healthy Spauge-Dawley male rats (average weight 160-210 g) were randomly divided to 3 groups (n=10). The animals in the first group received the same volume of vehicle for 50 days, and served as control. The second group received daily subcutaneous injection of 0.02 mg nicotine and daily oral gavage with 2 ml 20% Ethanol simultaneously. The third group was intact group, received no intervention and served to evaluate probable age changes in the reproductive system and to consider any stress induced by the treatments. For Sperm analysis, Cauda epididymis and vas deference were placed in 5 ml α-MEM supplemented with 2% BSA and cut into several fragments to allow the spermatozoa come out from reproductive ducts. Samples were incubated for 15 min. at 37○C and the following experiments were carried out on sperm suspensions in various groups. Sperm quality was determined by three parameters; concentration, motility and viability. The viability was assessed by three methods; Eosin exclusion dye test, Hypo-osmotic swelling test and Propidium iodide exclusion test.
Results: Sperm concentration was comparable between intact and control groups while, a reduction in sperm concentration was observed in ethanol/nicotine group compared to both intact and control groups ($p<0.01$) Motility was also significantly ($p<0.01$) impaired in ethanol/nicotine group compared to control group. Sperm viability was near to significant ($p=0.51$ and $p=0.063$ for HOS and PI tests respectively) altered following treatment with ethanol / nicotine.
Conclusion: According to our results, chronic co-administration of alcohol and nicotine severely alters the sperm quality.

Keywords: Alcohol, Nicotine, Sperm Parameter

P-23: The effect of Duration of Sheep Oocyte In Vitro Maturation on Induction of Parthenogenesis Using Ionomycin and 6-DMAP

Bahiraee A*, Shirazi A, Ahmadi E, Nazari H, Ahmadi E, Nazari H, Borjian S

Embryology Department, Shahrekord University, Shahrekord, Iran

Objective: The aim of this study was to compare the effect of time of partenogenetic activation (22 h versus 27 h after IVM) on subsequent development of IVM-derived ovine oocytes using either single activation agents (ionomycin 5 μM for 5 min) or combined activation treatments (ionomycin with 6-DMAP, 2.0 mM for 3 h). Cumulus oocyte complexes (COCs) were recovered from abattoir-derived ovaries and matured in vitro. After maturation, cumulus-free oocytes were activated according to the experiment designs. Activated oocytes were cultured in vitro in modified synthetic oviductal fluid medium and assessed for the cleavage, blastocyst, and hatching rates. Compared with IVF controls, the oocytes activated after 22 h of IVM, using only ionomycin, had a reduction in cleavage, blastocyst and hatching rates, whereas the addition of the 6-DMAP, an MPF-inhibitor, especially to oocytes activated after 27 h of IVM, restored development, measured by cleavage and hatching rates. However, the addition of 6-DMAP did not cause any positive effect on cell density/allocation between parthenote groups. Nonetheless, irrespective of the activation protocol, development to the blastocyst stage, total cell number and cell allocation (ICM/total cells) were significantly lower in parthenogenetic embryos than controls. In conclusion, compared with 22 h of IVM, it seems that a prolonged IVM time (27 h) and the addition of 6-DMAP improve embryo developmental potential of ovine parthenogenetic embryos.
Materials and Methods: Materials and methods Except where otherwise indicated, all chemicals were obtained from the Sigma (St. Louis, MO, USA). Oocyte collection and in vitro maturation The method for in vitro production of sheep embryo with slightly modification was the method of Thompson et al (1995). Oocytes used in this study were recovered from prepubertal and adult ovine ovaries collected at a local slaughterhouse and transported to the laboratory within 2 to 3 h in normal saline at temperature between 25 and 35 °C. Ovaries were washed 3 times with prewarmed fresh saline (37C), and all visible follicles with a diameter of 2 to 6 mm were aspirated using gentle vacuum (30 mm Hg) via a 20 gauge short beveled needle connected to a vacuum pump. The follicle content released in preincubated hepes-modified TCM, supplemented with 50 IU/ml heparin. The cumulus–oocyte complexes (COCs) with at least 3 layers of cumulus cells, oocytes with a uniform granulated cytoplasm, homogenous distribution of lipid droplets in the cytoplasm were selected for the experiments. Before culturing, oocytes were washed in Hepes-buffered TCM199 (H-TCM199) supplemented with 5% FBS (Fetal bovine serum, Gibco 10270), and 2mM glutamine. The oocyte culture medium (OCM) consisted of bicarbonate-buffered TCM 199 with 2 mM

L-glutamine supplemented with 0.02 mg/ml cysteamine, 1 IU/ml hCG, 1 µg/ml E2, 100 µl/ml penicillin, 100 µg/ml streptomycin, 10% FBS (Fetal bovine serum, Gibco 10270), and 0.2 mM Na-Pyrovate. The COCs were randomly distributed in maturation droplets (10 oocytes in 50 µl) and covered by sterile paraffin oil in a 60-mm Petri dish (Falcon 1008; Becton & Dickinson, Lincoln Park, NJ) and were then incubated under an atmosphere of 5%CO2 -95% air with 100% humidity at 39 ?C for 22 and 27 h. Preparation of sperm and in vitro fertilization The matured COCs were washed four times in H-SOF (HEPES- Synthetic Oviduct Fluid) solution and once in fertilization medium (SOF enriched with 20% heated inactivated estrous sheep serum) and then placed in 45 ?l droplets (10 COCs per droplet) of fertilization medium. Fresh semen was collected from a Lori-Bakhtiari breed ram of proven fertility. For swim up, 80-100 ?l of semen was kept under 1 ml of BSA-HSOF in 15 ml conical Falcon tube at 39C for up to 45 min. After swim up, the 700-800 l of the top fluid were then added to 3 ml of BSA-HSOF, centrifuged twice at 200 × g for 3 min and the final pellet was resuspended with BSA-HSOF. A 5 l aliquot of sperm suspension, were added to the fertilization drops at a concentration of 1 × 106 sperm/ml, Incubation was carried out at 39 °C in 5% CO2 in air with saturated humidity for 22 h. Twenty two hours after insemination, presumptive zygotes were denuded of surrounding cumulus cells by vortexing in H-FOS and transferred to culture droplets. Activation of oocyte Methods for activation of oocytes were modified from Susko-Parrish et al. (1994). After IVM (22 h and 27 h), cumulus cells were removed by incubation in 0.1% hyaluronidase in H-SOF at 38.5 °C for 2 min followed by vortexing for 3 min. Denuded oocytes were randomly allocated into single or combined treatment groups. Oocytes were treated with single activation agents, including ionomycin (5 M for 5 min. After 5 min exposure to ionomycin the oocytes were then rinsed in H-SOF containing 30mg/ml BSA to stop activation. All of the chemicals for oocyte activation were dissolved in H-SOF medium supplemented with 1 mg/ml bovine serum albumin (BSA). For the combined treatment, oocytes were firstly activated with the same concentrations of ionomycin as in the single treatment and then were immediately incubated in 2.0 mM 6-DMAP for extra 3 h. Following activation, oocytes were washed twice with the H-SOF medium and transferred to the culture medium. In vitro culture Presumptive zygotes in IVF groups and activated oocytes in parthenogenetic groups were allocated to 20 l culture drops (five to six embryos/drop) consisting of SOF supplemented with 2% (v/v) BME-essential amino acids, 1% (v/v) MEM-nonessential amino acids, 1mM glutamine and 8 mg/ml fatty acid free BSA. Embryo culture took place under mineral oil in a humidified atmosphere of 5% CO2, 7% O2 at 39 C. On the third and fifth day of culture (Day 0 defined as the day of fertilization) 10% charcoal stripped fetal bovine serum (FBS) was added to the medium. The percentage of cleaved embryos at day 3 and the percentage of blastocysts at day 7 were expressed on the basis of the number of oocytes at the onset of culture, and the percentage of hatched blastocysts at day 8 expressed on the basis of the total number of blastocysts present at day 7. The summary of experimental groups is presented in the below. Each experiment was consisted of at least 5 replicates. Group IVF: The in vitro matured oocytes for 22 h and 27 h were fertilized with fresh semen as control. Group Io: Denuded oocytes activated with 5 µm Ionomycin for 5 min. Group Io + 6-DMAP: Denuded oocytes activated with 5 µm Ionomycin for 5 min + 2 mM 6-DMAP for 3 h Differential staining Differential staining of inner cell mass (ICM) and trophectoderm (TE) compartments was carried out on day 7 blastocysts. Briefly, blastocysts were incubated in Triton X-100 prepared in the base medium (H-SOF containing 5 mg BSA/ml) for 20 seconds. The blastocysts were then stained in the base medium containing 30 µg/ml propidium iodide (PI) for 1 min. After two washes in the base medium, the blastocysts were transferred in ice-cold ethanol containing 10 mg/ml Hoechst 33342 for 15 min. The blastocysts were directly mounted into the small droplet of glycerol on glass slide and examined under an epifluorescent microscope (IX71 Olympus, Tokyo, Japan). ICM nuclei appeared blue, caused by DNA labeling with the membrane permeable Hoechst 33342, and trophoblastic cells appeared red due to staining of nuclear DNA with the membrane impermeable PI.

Results: Results Effect of oocyte age on effectiveness of parthenogenetic treatments on the development of parthenotes As shown in Table 1, two maturation times were considered to compare the effect of four activation regimens on parthenogenesis of ovine oocytes matured in vitro. The cleavage rates of artificially activated oocytes after 22h of culture, in group receiving either Io was lower than groups receiving Io + 6-DMAP. The cleavage rate in combined treatment groups (Io + 6-DMAP) was comparable with IVF group. There was no significant difference between IVF and oocytes artificially activated after 27h of culture in term of cleavage rate. The cleavage rates were significantly increased in groups Io when the maturation time was increased from 22h to 27h. The balstocyst rates of artificially activated oocytes after 22h of culture, in group receiving either Io was lower than groups receiving Io + 6-DMAP. The blastocyst rates in groups receiving Io + 6-DMAP after 27h of culture was higher than groups receiving either Io. The corresponding rate in IVF group, however, was significantly higher than parthenogenetically activated oocytes at both 22h and 27h of culture. The blastocyst rates, likewise cleavage rates, were significantly increased in groups Io when the maturation time was increased from 22h to 27h. The hatching rates in combined treatment groups after both 22h and 27h of culture were significantly higher than single treatment group. The corresponding rate, however, was significantly higher in IVF group compared to artificially activated oocytes after 22h of culture. The hatching rate

was significantly increased in group Io + 6-DMAPas the maturation time was increased to 27h.
Conclusion: it seems that compared with 22 h of IVM, a prolonged IVM time (27 h) and the addition of 6-DMAP improve embryo developmental potential of ovine parthenogenetic embryos

Keywords: Sheep, Parthenogenesis, Activation, Blastocyst

P-24: Effect of Hydrostatic Pressure Exposure to Sperm on In Vitro Fertilization

Bahrami Kamangar P[1]*, Azadbakht M[1], Amini A[1], Amiri I[2]

1. Department of Biology, Faculty of Siences Razi University, Kermanshah, Iran
2. Department of Anatomy, University of Medical Science, Hamedan, Iran
Email: pbkaman@yahoo.com

Objective: *In vitro* fertilization is an effective treatment to assist infertile couples. Sometimes infertility is because of male factors. Different factors have effect on sperm and causes infertility. Sperm from formation in male reproductive system until conception in female reproductive system is exposed to different factors. Increase or decrease in normal range of these factors causes damage on sperm and may causes infertility. Hydrostatic pressure is a crucial component of cell environment and fundamental physical quantity; also it is the main factor of integrity and function of cells. Pressure variation disorders, beyond physiological limits, may lead to infertility and subfertility. Any prejudiced about fertility or subfertility is based on results of sperm vital parameters including viability, motility and morphology. In this study, we examined the effect of hydrostatic pressure exposure to sperm on in vitro fertilization.
Materials and Methods: In this study we used female NMRI mice with 6-8 weeks old and male mice with 8-12 weeks old. The females were superovulated by 10 IU intraperitoneal injection of PMSG followed by 10 IU HCG, 51 h later. The females were sacrificed by cervical dislocation 16 h after the HCG injection and their oviducts removed and then MII oocytes were transferred into a plastic culture dishes containing HTF medium. Sperms separated from the epididymis and maintained in the Ham's F-10 culture medium supplemented with 10% FBS. Sperm suspension in the experiment group were placed within pressure chamber and pressurized into 100 mmHg for 4 h. Sperm vital parameters including morphology, viability and motility were evaluated and compared with control group. Then in vitro fertilization rate were studied.
Results: Our results showed that hydrostatic pressure reduced sperm viability ($p<0.05$). It induced abnormalities in morphology and reduced motility in sperms ($p<0.05$). Fertilization was occurred in two groups and pronucleus formation were observed in two groups but in experiment group development of embryos stopped at the first level of development.
Conclusion: Decrease in developmental rate of embryos form experimental groups may be related to damage in sperm after exposure to hydrostatic pressure.

Keyword: Sperm, Hydrostatic Pressure, IVF, Mouse

P-25: Simultaneous Effects of Herbal Extracts "Crocin and Oxalate" on Both Sex for Sex-Selection

Bakhtiyary M*, Alipanahzadeh H, Kazmirad S

Embryology Department, Iran University, Tehran, Iran
Email: s_anirash@yahoo.com

Objective: Sex-Selection
Materials and Methods: In male rat 20 microgram/ml crocin injected Intraperitoneally and after 2 month semen analysis carried out . In female rat 20 mg/ml oxalate have injected Intraperitoneal .ovulation time determind and female rat allowed for mating ,after 3 weak sex determination carried out in offsprig
Results: In experiment male rat spermatogenesis increased significantly . In experimental female rat cervical ph raised . we observed that 89%of offspring were male and 11% were female
Conclusion: Simultaneous use of crocin and oxalate results in increased male offspring

Keywords: Crocin, Cervical ph, Offspring, Spermatogenesis

P-26: Effects of Two Different Diluents on the Buffalo Epididymal Sperm Cryopreservation

Barati F[1]*, Khaksary Mahabadi M[2], Mohammadi Gh[1]

1. Clinical Sciences Department, Shahid Chamran University, Ahvaz, Iran
2. Basic Sciences Department, Shahid Chamran University, Ahvaz, Iran
Email: baratif@scu.ac.ir

Objective: Epididymal sperm (EP) have been successfully used for AI and in vitro production of embryos (IVP) to produce offspring in several of species, so it is necessary to store EP varying period of times. Influences of different types and concentrations of cryoprotectants, additives to freezing medium and determination of equilibration time have been investigated for EP cryopreservation. The citrate base, tris based and lactose based diluents for alpaca EP freezing. AndroMed®, a chemical defined medium, and Triladyl™, an egg yolk based medium, with an optimal equilibration time of 4 h were used successfully for cryopreservation of African buffalo (Syncerus caffer) EP. The Objectives of this study was to compare the influences of two basic semen extenders on post thaw epididymal sperm (EP)

viability and progressive motility.
Materials and Methods: Abattoir collected buffalo testicles (n=32) were allotted to three groups for cryopreservation. Caudal epidydimis dissected and aliquots of isolated EP analyzed for sperm parameters (viability and progressive motility) and splited to equal parts for cryopreservation. The cryopreservation performed with two different cryoprotective media: whole cow milk-7%glycerol (MG) or egg yolk-tris-citrate-7%glycerol (EYG) based on two step cryopreservation procedure. Pre freeze and post thaw percentages of progressive motile and viable sperms evaluated. Data expressed as Lsmeans±SEM.
Results: The percentage of unstained sperms decreased ($p<0.05$) after cryopreservation with MG (46.3 ± 3.4) and EYG (22.6 ± 4.2) compare to prefreeze sperm viability (91.1 ± 5.6). The percentage of motile sperms decreased ($p<0.05$) after cryopreservation with MG (16.5 ± 2.6) and EYG (11.6 ± 2.5) compare to prefreeze sperm motility (73.4 ± 3.4). MG protected viability and progressive motility of EP against cold shock more efficiently than EYG ($p<0.05$).
Conclusion: Cryopreservation has adverse effect on buffalo EP. MG diluent is superior to 20% level of egg yolk plus glycerol on buffalo EP freezing output. More studies must do to find the optimized protocol for cryopreservation of river buffalo EP.

Keywords: Epididymal Sperm, Cryopreservation, Buffalo

P-27: The Effect of Ethanol on Growth and Development of Two-Cell Arrested Mouse Embryo

Darabi M.R[1]*, Shiravi A.H[2], Nezhadi A[2], Rafiei M[1]

1. Department of Anatomy, Arak University of Medical Sciences, Arak, Iran
2. Department of Biology, School of Basic Sciences, Azad University, Damghan, Iran
Email: international@royaninstitute.org

Objective: Stirility is a problem throughout the world. Decreasing the growth and developmental rate of embryo and arresting in certain step of development like two cell block, could be the reason of infertility in some couples. Previous study show that arrest and retardation in embryo development can produced by low temperature exposure. We aimed to evaluate the effect of Ethanol on growth and development of mouse two-cell arrested embryo.
Materials and Methods: The 4-6 week old female mice were coupled with male mice following superovulation and positive vaginal plaque mice were killed 48 hour after HCG injection by cervical dislocation method. Two cell embryo were collected in RPMI medium and divided and cultured (in M16 medium) in three groups. The 2nd and 3rd groups were exposed to 4°C for 24 hour in order to delay and arrest for cleavage and developmental rate. The 2nd group (2nd control) were incubated immediately, while the 3rd group (experiment) were exposed to % 0.1 Ethanole for 5 minutes and the 1st group (1st control) without any exposure to low temperature group were incubated .
Results: The data analysis by one-way ANOWA show that the developmental rate of embryos exposed to low temperature (4°C) significantly decreased ($p=0.001$), retardation and arrest being produced. The mean of cleavage rate between groups were not significantly affected, but the mean percent of degenerated embryos between groups have significant differences ($p=0.045$). On the other hand the mean percent of morulla is significantly different between groups ($p=0.005$) similarly the mean percent of blastocyst and hatched blastocyst have significant differences between groups ($p=0.014$) ($p=0.001$) after 120 hr evaluation.
Conclusion: Effect of %0.1 Ethyl-alchol on arrested two cell embryos can significantly increase the mean percent of morulla and development up to blastocyst and hatching blastocyst stage related to control group, without any significant effect on cleavage rate.

Keywords: Ethanol, Mouse, Embryoe, Infertility, Cleavage, Blastocyst

P-28: Maternal Lead Acetate Exposure Affects Postnatal Development of Ovarian Follicles in Wistar Rats

Dorostghoal M*, Moazedi, Moattari M

Biology Department, Shahid Chamran University, Ahvaz, Iran
Email: mdorostqoal@yahoo.com

Objective: During the last decades, the environmental contamination by lead generated by human activities has become an evident concern. So, effects of lead acetate were studied on histomorphometric structure of ovary during postnatal development in Wistar rats.
Materials and Methods: The female pups randomly divided in control and experiment groups. The mothers of experiment groups received 100 and 300 mg/L/day lead acetate via drinking water during lactation. The ovaries of pups were removed at 3 months after birth, their weights recorded and were fixed in Bouin's solution. Followed tissue processing, 5-6 µm paraffin sections were stained with haematoxylin-eosin, and then, number, diameter and surface area of primordial, primary, secondary, graffian and atretic follicles were estimated in control and experiment groups.
Results: The results showed that the means of ovary weights in low and high doses groups decrease at 3 months after birth in comparison with control group. Microscopic results indicated that number of primordial and atretic follicles increased significantly ($p<0.05$) at puberty in low and high doses groups in comparison with control group. Also, mean diameter and surface area of secondary and graffian follicles decreased significantly ($p<0.05$) in high dose group.
Conclusion: Present study shows that lead acetate af-

fect prepubertal ovarian follicle development and can decrease fertility and reproductive efficiency of female Wistar rats.

Keywords: Ovary, Postnatal Development, Fertility, Lead Acetate

P-29: Protective Effect of Melatonin on Myleran-induced Changes in Testicular Damage and Sperm Characteristics in Mice

Faghani Langroudi M[1]*, Ghasemi F.M[2], Nasiri E[2], Bahadori M.H[2]

1. Histotecnique Lab, Guilan University, Rasht, Iran
2. Embryology Lab, Guilan University, Rasht, Iran
Email: mfaghani2000@gmail.com

Objective: The aim of this study was to investigate the possible protective role of melatonin on myleran induced spermiotoxicity using quantitative histopathological approches and study of sperm parameters.
Materials and Methods: Male adult NMRI mice were divided into four groups. The control group received physiological saline; animals in group A received a single dose of myleran 20 mg/kg intraperitoneally. Animals in group B were treated with melatonin 10 mg/kg intraperitoneally. Melatonin was administered for 5 days. Group C received a 5 day of melatonin following administration of myleran. 35 days after the treatment all animals were killed and the traits of sperm characteristics, testicular histopathological and quantitative findings were determined.
Results: Myleran decreased sperm concentration ($p<0.001$), sperm motility ($p<0.01$) and increased abnormal sperms ($p<0.001$) as compared with the control. While melatonin caused a marked normalization in sperm parameters in group C. Seminiferous tubules showed reduction in diameters ($p<0.01$) and in germ cells numbers ($p<0.001$) in myleran treated group. However administration of melatonin in group C increased seminiferous tubules parameters.
Conclusion: Although the mechanism is not clear, the results from this experimental study suggest that the melatonin have a possible protective effect against myleran- induced testicular damage, probably by decreasing oxidative stresses.

P-30: Semen Analysis of Men with Infertility Disorders

Hafezi Ahmadi M.R[1]*, Delpisheh A[2], Peyman H[3], Yasemi M[3], Khodadadi I[3], Seydmohamadi A[3], Sahraie A[3], Azizyanfar M[3], Zaryan A[3]

1. Pathology, Ilam University, Ilam, Iran
2. Epidemiology, Ilam University, Ilam, Iran
3. Medical Student, Ilam University, Ilam, Iran
Email: nu85_hadi@yahoo.com

Objective: Semen analysis is considered as a first step in diagnosis and assessment of infertile men.
Materials and Methods: This is a case-control study of 42 men with infertility disorders in Ilam western of Iran. Semen analysis was done using Weili dynamic sperm analysis software according to WHO classification. A standard questionnaire was used for collecting demographic characteristics.
Results: Based on semen analysis, research participants were stratified into two groups. The case group, "Oligospermia" with sperm counts of less than 20 million in mL (n=12) and the control group "Nonoligospermia" with values above that cutoff point (n=30). Mean age for cases and controls were 29.1± 4. 5, and 32.4± 5.4 years respectively. Almost all cases (91.7 %) had problems in the class A in which means sperm move ahead slowly. The corresponding rate for the control group was 70.0%. Only 8.3% of case group had normal live ratio (L.R) whilst this rate was 40% in controls. There was a significant difference between cases and controls in terms of family history of infertility disorders ($p=0.001$). Having a military job was also another significant difference between cases and controls ($p=0.001$).
Conclusion: Early semen assessment in high risk men had a significant role in infertility process and helps to prevent of its severe complications.

Keywords: Semen Analysis, Infertility, Weili Software, Ilam

P-31: Effect of Pomegranate Juice (Punica Granatum L.) Consumption on Sperm Parameters and Fertility Potential in Mice

Hasani Tabatabaee S.S*, Amini Rad O, Khalili M.A, Soltani H.R

Andrology, Yazd Fertility and Infertility, Yazd, Iran
Email: omid_amini2007@yahoo.com

Objective: sperm fertilization potential depends on factors such as sperm count, motility and morphology. A variety of factors, including free radicals, may disturb the sperm parameters. Elements such as vitamins C & E and polyphenols have anti-oxidental effects. Since, sperms are poor in combating with free radicals; the aim of this study is to determine the effect of pomegranate juice (PJ) on sperm parameters (count, morphology, motility) and fertility potential in mice.
Materials and Methods: in this experimental study, 20 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=10) and experimental group (n=10) randomly. The experimental group received 20% pomegranate juice for 1 month (duration of spermatogenesis). We took one generation from each group to study the fertility rate. After autopsy , a sample from the tail of epididymis region was taken to test the

sperm parameters by light microscope. SPSS software version 14.0 and mann-whitney u test was used
Results: The results showed that PJ consumption increased sperm count from (34.2 ± 15.1) in control group to (49.7 ± 13.6) in case group (p=0.014), also, the rate of non progressive motility in case group decreased when compared with control group (p=0.007). In addition, the normal morphology of the case group improved significantly (p=0.001). The rate of fertility potential increased from 5.5 ± 3.3 in controls to 10.0 ± 1.3 in case mice (p=0.007).
Conclusion: PJ is able to improve the quality of sperm parameters, as well as fertility potential in mice. Probably, intake of this antioxidant by infertile men improves the quality of their sperm parameters.

Keywords: Juice, Sperm, Fertility, NMRI Mouse

P-32: Effects of Repeated Subsquent Gonadotropin Adminatration on Endometrium Diameter and Ovarian Homoral Response

Heidari F[1]*, Sadrkhanlou R[2], Rastegarnia A[1], Ahmadi A[2], Ghafari S[2], Raberi A[2], Kargar K[2]

1. Veterinary Medicine, Islamic Azad University, Urumia, Iran
2. Basic Sciences, Faculty of Veterinary Medicine, Urumia, Iran
Email: farshadheidari@yahoo.com

Objective: Gonadotropin has been widely used word wide for many years to induce ovulation .three main exogenous gonadotropins are used for follicular development and ovulation induction –follicle stimulating hormone(FSH),luteinizing hormone(LH)and human chronic gonadotropin(hCG).Currently ,these gonadotropins or their recombinant are present in the urine(with the exception of (LH).Other authors have reviewed the physiology of the ovulatory cycle and the role of the gonadotropin in ovulation induction in patient with anovulatory disorders and in multi-follicular development for assisted reproductive technologies. The aim of this experiment is to find the optimal subsequent time for repeated gonadotropin administration for the maximum response for ovarian follicular development
Materials and Methods: Twenty five female mature Syrian mice of 6 weeks old were used to determine the optimal subsequent period for repeated gonadotropin administration for the raising of ovarian follicular estrogen level and measurement of the endometrioum and epithelial layer thickness and gland dispersion.The animals were housed at animal house with 12hours light 12 hours dark periods daily at temperature 22 with free access to food and water adlibidum.The animals were divided in to 5 groups, all groups received 10 (IU)PMSG(folligon) IP and following 48 hours of hormone treatment the first group of animal(check) was sacrificed and the blood was collected for estrogen assay and uterine horns were dissected and processed for tissue embedding and H&E staining to evaluate with hormonal response.The other four groups were injected 10 IU PMSG at 2,4,6,and 8 week later subsequently and their plasma estrogen level were measured by electrochemilumiescenc(ECL)assay technique
Results: The result of this experiment has shown that beginning from the third group, the level of estrogen hormones in blood plasma and endometrium and epithelial layer thickness together with gland dispersion that was measured by light microscope was raised at its maximum amount and remained constant at the two other groups which were significantly higher than the control groups.
Conclusion: This experiment has shown that the optimal time following first gonadotropin treatment cancellation for the next gonadotropin administrated is four weeks after first hormone treatment with regarding to the estrous cycle period in the mice which is about 4-5 days. The optimal time for repeated subsequent gonadotropin administration for ovarian follicular development is about four weeks from former PMSG injection

Keywords: Gonadotropin, Estrogen, Ovary, ECL.Endometrium

P-33: Follicular Fluid Sellective Hormones and Cytokines Concentration and Relationship with Embryo Implantation in Women Undergoing Intracytoplasmic Sperm Injection Method in Motahhari Hosp. Urmia

Ilkhanizadeh B[1]*, Nahali Moghaddam A[1], Hajshafieha M[2], Mahzad Sadaghiani M[2], Salami S[3]

1. Pathology, Urmia Medical Sciences University, Urmia, Iran
2. Infertility, Urmia Medical Sciences University, Urmia, Iran
3. Biochemistry, Urmia Medical Sciences University, Urmia, Iran
Email: drnahali@gmail.com

Objective: Concentrations of certain substances in follicular fluid (FF) may related to fertilization outcome. The study aim was to identify FF markers with which to predict embryo implantation potential.
Materials and Methods: Concentrations of selected hormones, cytokines and growth factors in FF samples obtained during assisted reproduction treatment, Intracytoplasmic Sperm Injection (ICSI) method were related with treatment outcomes.
Results: Mean concentrations of 17 beta-estradiol (E2), prolactin (PRL) and insulin-like growth factor (IGF)-I were significantly higher, in treatment attempts leading to a clinical pregnancy as compared with those in which no pregnancy was established. LH, growth hormone (GH), progesterone, and interleukin-1 (IL-1) concentrations are similar in successful and unsuccessful attempts.
Conclusion: Fertilization outcomes were related to FF levels of PRL, E2 and IGF-I. But LH, GH, progesterone, and IL-1 are not markers of success.

Keywords: Implantation, Hormone, Cytokine, Follicular Fluid, ICSI

P-34: Study of Effects of Morphine on Light Microscopic Structure of Uterus

Jafarpur M

Mashhad University of Medical Sciences (MUMS), Mashhad, Iran
Email: jafarpurmokhtar@yahoo.com

Objective: So opiate consumption has unfavorable consequences on different organs, we decided to evaluate the effects of morphine on the uterus structure. the present study focused on the probable effects of morphine on histological structure of uterus in Balb/c mice.
Materials and Methods: Forty Balb/c female mice were provided for this study. Each three females mice were crossed with one male and vaginal plaque was considered as zero day of pregnancy (E0). Nonpregnant mice were displaced by other females with vaginal plaque. The pregnant females were divided into four groups(two experimental and two control groups). Five mg/kgw for experimental group number1, and ten mg/kgw for experimental group number2 morphine were injected intraperitonealy (IP) (every day during 15 days) in each experimental group, respectively. The same volume of saline was used for IP injection in the first control group and the other control group did not receive any injection. In the fifteenth day of gestation (E15), the pregnant females were sacarified and their uteri were removed. After tissue processing hematoxylin-eosin staining was done and all of the samples were studied using light microcopies.400sections from 40 mice were provided and microscopically scrutinized for finding the changes such as cell infiltration, vascular congestion and necrosis. Data was analyzed based on chi square and fisher tests.
Results: In light microscopy, our data showed the uteri of control groups were normal, but in experimental groups, our findings were some picnotic cells, inflammatory cells infiltration and congestion of blood vessels. The percentage of inflammatory cells infiltration and picnotic cells were 60% in the first experimental group,70% in the second and 0% in both the first and second control groups. The percentage of blood vessels congestion in the experimental groups (1 &2) was 70 % and 0% in the control groups(1&2). according to our findings There was a significant difference between the experimental and control groups (p=0.0001).
Conclusion: data indicated the morphine administration caused histologic lesions that may be responsible for abortion or infertility.

Keywords: Mouse, Morphine, Uterus, Histological Disintegration

P-35: Adverse Effects of Hydrostatic Pressure on Sperm Vital Parameters

Karimi N[1]*, Kamangar P[1], Azadbakht M[1], Amini A[1], Amiri I[2]

1. Biology Razi University, Kermanshah, Iran
2. Anatomy, Hamedan Medical Science, Hamedan, Iran
Email: narin_elegant@yahoo.com

Objective: Studies indicated that 50 percent of infertility in couples is related to males. Prejudiced about fertility or subfertility based on results of sperm vital parameters including viability, morphology and motility. In males different parameters are identified as cause of infertility and subfertility, such as environmental causes including: physical-mechanical and sperms own causes including: reduce of sperm number, abnormality in morphology and motility and changes in the sperm chromatin condensation. Hydrostatic pressure is a crucial component of cell environment and fundamental physical quantity; also it is the main factor of both integrity and function of cells. Sperms exposed to different range of hydrostatic pressure within male reproductive system and after fertilization and enter to the female reproductive system. Pressure variation disorders, beyond physiological limits, may lead to infertility and subfertility .This study examined the effects of pathological range of hydrostatic pressure on sperm in aspect of survival, morphology and motility. The objective of this study was to develop and understanding of the link between abnormalities in sperm that induced by hydrostatic pressure in relation to reduce of male fertility
Materials and Methods: Sexually mature NMRI male mice, ages of 8-12 weeks-old were as sperm donors. Mouse sperms separated from the cauda epididymis and maintained in Ham's F-10 culture medium supplemented with 10% FBS and divided into two groups as control and experiment. Sperm suspension in the experiment group were placed within pressure chamber and pressurized into 100 mmHg for 2 and 4 h, sperm vital parameters were evaluated and compared with control group.
Results: Our results showed that hydrostatic pressure changed sperm viability gradually and reduction happened significantly after 4 h ($p<0.05$). It reduced sperm motility ($p<0.05$) and increased abnormalities in sperm morphology ($p<0.05$).
Conclusion: Hydrostatic pressure as a physical-mechanical stress factor affects sperm vital parameters and may cause male infertility or subfertility as a result of changing in sperm parameters

Keywords: Hydrostatic Pressure, Sperm, Vital Parameters, Mouse

P-36: Effects of Acrylamide on Membrane Integrity and Sperm Parameters in Mice

Kermani M[1]*, Anvari M[2], Talebi A[2], Amini O[1], Mires-

maili M[3]

1. Meddical Student, Shahid Sadoughi University, Yazd, Iran
2. Embryology, Research Center for Infertility, Yazd, Iran
3. Laboratory, Research Center for Infertility, Yazd, Iran
Email: mimi_lookluck@yahoo.com

Objective: Acrylamide is a chemical reactive substance used in various industries such as polymer industry, cosmetic, adhesives, paper and textile industries ,wastewater treatment, and laboratory gels . Recently, the discovery of acrylamide in a variety of human foods like heat-processed starchy foods such as potato chips, French fries, and bread was reported.Acrylamide is known as a carcinogen and cytotoxic material,so the objective of this study was to determine the effect of acrylamide on membrane integrity and sperm parameters in mice.
Materials and Methods: In an experimental study, thirty male NMRI mice, on age 8 to 10 weeks and weight in 25-30 gr were randomly allotted into three groups that each one had 10 mice. Group I and Group II were fed on water solutions containing acrylamide 5 and 10 mg/kg/day for eight consecutive weeks, while the third group on fresh water only as the control.
Results: In sperm analysis the total motility like fast motility and slow motility in both Group I and II were decreased significantly ($p = 0.00$), but no significant change was observed in non-progressive motility ($p > 0.05$). Membrane integrity of the tail of sperms in both Group I and II had significant decrease ($p = 0.00$) but membrane integrity of the head of sperms,decreased significantly only in Group II,as in sperm count ($p < 0.05$).Sperm morphology was not significantly changed ($p > 0.05$).
Conclusion: These results indicate that acrylamide by effect on membrane integrity decreased sperm viability,also causes abnormal sperm parameters.

Keywords: Acrylamide, Mebrane Integrity, Spermatogenesis, Mouse

P-37: Melatonin Improves Quality of Seminiferous Tubules and Sperm Characteristics of Acetyl Salicylate Acid Treated Male Mice

Khajeh Jahromi S[1]*, Ghasemi F.M[1], Hedayati M[1], Hemadi M[2]

1. Histotechnique Lab, Anatomy Department, GUMS, Rasht, Iran
2. Anatomy Department, Faculty of Medicine, Ahwaz, Iran
Email: sina_sayarehbozorg@hotmail.com

Objective: Melatonin is a potent physiological scavenger of hydroxyl radicals. In the present study we aimed to demonstrate the effect of melatonin on testicular damage and sperm parameters deficiency induced by Acetyl salicylate acid(ASA), in adult male mice.
Materials and Methods: Male NMRI mice were divided into 4 groups: 1) control 2) ASA treated group 3) melatonin treated group 4) Melatonin-ASA treated group. ASA were administered in dose of 160mg/kg orally for 14 days. Melatonin were administered in dose of 10 mg/kg for 5 days intraperitoneally. Control mice were received vehicle (phosphate buffer) orally. The animals were sacrificed and their testes dissected 15 days after the treatment. Evaluations were made by determining Johnson's score, epididymal sperm content, and sperm morphology and testis volume. Statistical analysis was performed by ANOVA test.
Results: ASA treated mice showed a reduction in Johnson's score, sperm content ($p<0.01$), normal morphology ($p<0.01$) and testicular volume ($p<0.05$) in compare to control. Melatonin in group 4, significantly increased maturation of seminiferous tubules and testicular volume ($p< 0.001$). Melatonin increased quality of quantity of sperm parameters in group 4 when compared to group 2.
Conclusion: These results suggest that intraperitoneal administration of melatonin for 5 days is a potentially beneficial agent to improve the quality of spermatogenesis and sperm parameters in testis damaged by ASA, probably by decreasing oxidative stresses.

Keywords: Melatonin, Acetyl Salicylate Acid, Spermatogenesis, Sperm Parameters

P-38: Phosphoproteomic Pattern of Sperm Cell in Normozoospermic and Infertile Teratozoospermic Men

Lakpour N, Hodjat M, Asgharpour L, Akhondi M.M, Jabbari S, Ardekani A.M*

Department of Biology, Islamic Azad University, Science and Research Branch, Tehran, Iran
Email: jabbari487@yahoo.com

Objective: Spermatozoa are specialized cells that remain inactive and must undergo posttranslational modifications such as phosphorylation for cellular processes, including capacitation. During capacitation, spermatozoa gain hyperactive motility, interact with zona pellucida (ZP) and undergo acrosome reaction. In this study we have investigated the protein tyrosine phosphorylation pattern of spermatozoa in normozoospermic and infertile teratozoospermic men referred to Avicenna Infertility Clinic in Tehran
Materials and Methods: Semen samples were collected and spermatozoa were isolated using percoll gradient centrifugation. Spermatozoa were then incubated for 6 h at 37ºC in 3% Bovine Serum Albumin- supplemented Haḿs-F10 for capacitation. Total proteins of spermatozoa were extracted following standard protocol. To characterize phosphorylated proteins, two dimensional gel electrophoresis (2DE) coupled with western blotting was performed.
Results: Our results from 2DE and western blotting showed that during capacitation, some proteins become phosphorilated in spermatozoa from normozoospermic men but not in teratozoospermic men
Conclusion: These findings suggest that compromised sperm protein phosphorylation in teratozoospermermic men can potationally be responsible for diminished ca-

pacitation and low fertilization success in this group.

Keywords: Capacitation, Tyrosine phosphorylation, Normozoospermic Men, Teratozoospermic Men.

P-39: Effect of Ovarian Follicle Isolation Technique on *In Vitro* Follicle Culture and Maturation

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

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

Objective: *In vitro* culture and maturation of immature ovarian follicles is an alternative for preserving the fertility potential of young persons using cryopreserved ovarian tissue. The aim of this study was to compare the effect of the mechanical and enzymatic isolation of follicles on the subsequent follicular and oocyte development.
Materials and Methods: Preantral follicles were isolated from immature NMRI mice ovaries either mechanically (group M) or enzymatically (group E) and cultured for 10 days. Growth, survival rates of the follicles were assessed during the culture period for both groups. Data were analyzed using the chi-square test.
Results: After 2 days of culture, basement membrane disruption had occurred in 36.7% of group M and 100% group E ($p< 0.05$). At the end of culture period the survival rate was higher in group M than in group E (74.3 and 63.55 respectively). Antral formation was observed in 29.5% of surviving follicles in group M and 43.1% of group E ($p< 0.05$).
Conclusion: The results indicated the mechanical isolation produces less damage to the preantral follicles than enzymatic isolation and could therefore produce better quality follicles.

Keywords: Isolation, Follicle, *In Vitro* Culture, Mouse

P-40: Effect of Ascorbic Acid on *In Vitro* Fertilization of Mouse Oocyte and Embryo Development to the Bastocyst Stage

Moayedi A[1]*, Niyazi E[1], Bahadori M.H[1, 2], Heidarzadeh A[3], Ahmadi M[1], Ahmadi M[1]

1. CMRC, Guilan Uniiversity of Medical Science, Rasht, Iran
2. Anatomy, Embryology Ward, Rasht, Iran
3. Community Medicine, Rasht, Iran
Email: aida_moayedi@yahoo.com

Objective: Infertility is a main problem of approximately 15 % of couples trying to conceive in a period of their lives. In the past decades in vitro fertilization and its related techniques have been an appropriate method to cure infertile patients. Studies have shown that in present in vitro conditions rate and speed of embryo development, cell number, their synthetic activity and their biological competence is less than those which developed in in vivo environment. One of its major causes is generation of reactive oxygen species (ROS) in in vitro cultures that results in degrees of infertility. Ascorbic acid is a kind of hydrophilic antioxidants in the follicular fluid that can scavenge oxygen metabolic like hydrogen peroxide, super oxide anions, and hydroxyl radicals from the environment. So, regarding above this study conducted to evaluate different concentrations of ascorbic acid on the improvement of embryo development till the blastocyst stage.
Materials and Methods: Two pronucleous (2 PN) zygotes were obtained from female NMRI mice after administration of i.p. injection of 5 IU Pregnant Mares Serum Gonadotropin (PMSG) and subsequent human Chorionic Gonadotropin (hCG) injection. Groups of 2 PN zygotes were randomly placed in T6 + BSA 16 mg/ml medium drop without or with ascorbic acid(100, 400) and halved doses(50, 200) supplemented at 24 and 48 hours they were cultured to the hatched blastocyst stage, and the number of embryo in different stage was recorded under an invert microscope and compared.
Results: In this study, Addition of Ascorbic acid to embryo culture media promoted the development from 2 PN stage embryos to morula, blastocyst and hatched blastocyst. The addition of ascorbic acid in 50ng/ml supplemented at 24 and 48 into the culture medium increased the percentage of 2 PN mouse embryos that developed into blastocysts and hatched blastocysts significantly, whereas in the presence of 100, 200 and 400 was not significantly higher.
Conclusion: Ascorbic acid plays an important role in the development of preimplantation embryo. It can promote embryo development

Keywords: Ascorbic Acid, *In Vitro* Fertilization, Bastocyst

P-41: Success Rate of ICSI and its Implicating Factors, in QOM Branch of Royan Institute, A Retrospective 5 Year Study

Moslemi MK[1,2]*, Ale Mohammad Amir A[2], Amiri K[2]

1. Urology Department, Qom Medical Sciences University, Qom, Iran
2. Infertility Center of Jihad Aaneshgahi, Qom Branch, Qom, Iran
Email: moslemi_urologist@yahoo.com

Objective: This is a retrospective study in which we evaluated ICSI results in our scheduled couples,defining demographic data and relating etiologic factors to the ICSI success
Materials and Methods: we evaluated retrospectively ICSI results in 400 couples that underwent this procedure in a 5 year period from the beginning of 2003 to the end of 2007.the infertility causes were; male causes and femal causes or idiopathic.like

,varicocel,idiopathic male subfertility,azospermia,ejaculation failure,cervical factor,endometrisis,tubal factor ,pelvic adhesions,hyperprolactinemia and secondary hypogonadism.for 400 cases a total of 436 cycles of ICSI induced.for the azospermic males we used PESA or TESE ,for sperm retreival.
Results: ICSI success per each case was 30% in our study,this rate was 27.5% per cycle.the success rate per each specific cause was as follows:ejaculation failure 33.3%, azospermia; 30%, male subfertility; 29.3%, varicocel; 30.1%, PCO; 30%, tubal factor; 23.9%, cervical factor; 25%, pelvic adhesions; 26.7%, endometriosis; 23.5% and hyperprolactinemia; 26.5%. success rate of ICSI with PESA was 27.4%, this rate was 6.7% for TESE,that was significant statistically.duration of infertility in positive ICSI cases was, 5.94 years,this rate was 7.58 years for negative cases.we have 27 twin pregnancy from 120 pregnancy(22.5%).
Conclusion: ICSI is a highly demanded,expensive and nearly invasive ART technique ,that its use must be individualized,and each case must be carefully selected, patiently.

Keywords: ICSI, ART, PESA, TESE, Sperm Retreival, Infertility

P-42: The Rate of IUI Success and its Effective Factors - in QOM Branch of Royan Infertility Center, A Retrospective Study

Moslemi MK[1,2]*, Ale Mohammad Amir A[2], Amiri K[2]

1. Urology Department, Qom Medical Sciences University, Qom, Iran
2. Infertility Center of Jihad Aaneshgahi, Qom Branch, Qom, Iran
Email: moslemi_urologist@yahoo.com

Objective: This is a retrospective study in which the rate of IUI success and its implicating factors is evaluated.
Materials and Methods: We evaluated 385 consecutive infertile couples that underwent IUI in our center ,from 2006 to 2007.in 313 couples we used concentration gradient technique,and in 72 couples swimming up method is used.the range of infertility duration was 1 to 8 years.the age range of males was 21 to 48 years ,this was 17 to 36 years for females.
Results: Regardless of infertility causes, total success rate of IUI was 16.6% per cycle and 24.7% per patient. mean inducted cycle in pregnanted females was 1.61 cycle.rate of IUI success regarding the cause, was as follows: tubal factor 36%, varicocel:29.3%,ejaculatory failure 27.5%,cervical factor 26.6%,ovulatory dysfunction 25%,pelvic adhesion 25%,idiopathic male subfertility 23.9%.hyper prolactinemia 22% hypothalamic amenoreha 25% and endometriosis 6.6%.success rate of concentration gradient method was 25.5% and with swimming up way it was 15.8%.mean duration of infertility in IUI positive cases was 3.43 years and this rate in IUI negative cases was 4.7 years ,that was statistically significant.
Conclusion: IUI is a safe near natural technique of conceiving a child that if used in its specific indications has perfect success rate.

Keywords: IUI, Infertile Couples, Concentration Gradient, Swim up Method, Infertility

P-43: Effect of Artemisia Absinthium Flowers Extract on Fertility in Male Albino Rats

Parandin R.A[1]*, Ghorbani R[2]

1. Biology Department, University of Payamenoor, Kermanshah, Iran
2. Anatomy Department, University of Medical Scince, Kermanshah, Iran
Email: rahmatparandin@gmail.com

Objective: Artemisia absinthium is a species of wormwood, native to temperate regions of Eurasia and Middle East.To evaluate possible fertility activity of Artemisia absinthium flowers extract in male rats.
Materials and Methods: 24 Male rats(70-90 days old) were randomely divided into 3 groups. Animals in group A(Control group) were administered with the distill water only. Animals in group B and C were administered with oil extract of A. absinthium flowers at the doses of 200 and 400 mg/kg/day for 50 days. At the end of 50 treatment period, Body weight, Epididymis weight, GSI(gonadosomatic index), sperm motility, sperm viability, ESR (epididymal sperm reserves), DSP(daily sperm production) and testosterone concentration were assessed. Fertility percentage was evaluated with mating test.
Results: There was a significant decrease in the GSI, sperm motility, sperm viability, ESR, DSP, testosterone concentration and Fertility percentage especially in higher dose.
Conclusion: Our data concluded that the extract of A. absinthium flowers at both doses(200 and 400 mg/kg) had a negative impact on the fertility parameters.

Keywords: Artemisia Absinthium, Fertility, Male Rat

P-44: Relevance of LIF and EGF on Mouse Preimplantation Embryo Development

Maryam Parvini[1]*, Iraj Amiri[2], Khadijeh Heidarbeigi[4]

1. Developmental Bioloby, Razi University, Kermanshah, Iran
2. Hamadan Medical Science University, Hamadan, Iran
3. Developmental Bioloby, Razi University, Kermanshah, Iran
4. Developmental Bioloby, Razi University, Kermanshah, Iran
Email: mpparvini@yahoo.com

Objective: Recent evidence suggests that Leukemia inhibitory factor (LIF),a member of interleukin-6 family has biological actions on preimplantation embryo development.Also it is established that Epidermal growth

factor(EGF) is a strong mitosis-promoting agent that improve the preimplantation embryo development by increasing the cell metabolism and proliferation. The purpose of the present study was to investigate the effects of these factors in combination together on preimplantation embryo development.
Materials and Methods: Six to eight weeks old NMRI mice were super ovulated by injection of 10IU PMSG and 10 IU hCG 46-48h later. The mated mice were killed 46 hours after hCG. injection, oviducts were flushed and two-cell embryos collected and divided randomly to four groups as following: Control), treatment 1 (LIF), treatment 2 (EGF), treatment 3 (LIF+EGF).In each group the embryos were cultured in an incubator at 37°C with 5% CO_2 and 90% humidity for 72h.The state of embryo development was evaluated in 24,36,48,60 and 72 hours following culture. At the end of culture, cell apoptosis was studied by the terminal deoxynucleotidyl transferas-mediated dUTP nick end-labeling(TUNEL) technique.
Results: Significant difference was detected in the rate blastocyst formation after 36 hours in the LIF and LIF+EGF groups ($p<0.05$). This difference was also seen in the rate of hatching ($p<0.05$) and average of total cell number ($p<0.05$) after 72 hours. In comparing the apoptotic index, there was no significant difference between the control and treatment groups
Conclusion: The findings in this study suggest a beneficial effect of LIF and EGF on blastocyst formation, hatching and its total cell number *in vitro*.

Keywords: LIF, EGF, Mouse, Preimplantation Embryo

P-45: premature LH Surge in Intrauterine Insemination (IUI) Cycles and GnRH Antagonist Rule

Pourmatroud E[1]*, Ghazizadeh S[2]

1. Vali-E-Asr Reproductive Health Research Center, Tehran, Iran
2. Tehran University of Medical Science, Tehran, Iran
Email: e.pourmatroud@yahoo.com

Objective: To assessment the usefulness of premature LH surge prevention in intrauterine insemination cycle by GnRH antagonist administration.
Materials and Methods: 60 patients with unexplained or mild male infertility or minimal to mild endometriosis, were enrolled to this prospective randomized controlled trial. 20 patients in group A (with GnRH antagonist) and 40 patients in group B (without GnRH antagonist) In all of participants CC+HMG were used for ovarian stimulation and when at least one follicle with ≥ 16 mm diameter was seen, LH surge checked by urinary LH kit; in patients with negative results, HMG was continued, but in group A 0.25 mg Ganirelix SQ was administrated for two days ,then in both groups HCG was injected on the third day and IUI was done 36-40 hours later. Pregnancy was the primary outcome.
Results: Base line characters and clinical parameters were similar in both groups except ≥18 mm follicles in group A (p value= 0.003). pregnancy rate in both group was not significantly different (10% in group A and 15% in group B).
Conclusion: At least in CC+HMG stimulated cycles for IUI, the occurrence of premature LH surge could be useful and GnRH antagonist administration could have an interventional mistiming rule.

Keywords: IUI, GnRH Antagonist, Urinary LH kit

P-46: The Effects of Electromagnetic Field on Mouse Blastocyst Apoptosis

Rajaei F[1]*, Borhani N[2], Shams A.R[2]

1. Infertility Centre, Qazvin University of Medical Sciences, Qazvin, Iran
2. Anatomy, Qazvin University of Medical Sciences, Qazvin, Iran
Email: farzadraj@yahoo.co.uk

Objective: The growing development of electronic industries and the increasing use of electrical appliances have led to higher rise in chronic exposure of people to extremely-low-frequency electromagnetic field (ELF-EMF). The aim of the present study was to investigate the possible effect of electromagnetic field exposure on DNA fragmentation in mouse blastocysts
Materials and Methods: Eighty female NMRI mice were randomly divided into 2 groups of 40 animals: Control group was left unexposed. EMF group was exposed to 50 Hz & 0.5 mT EMF for 4 hours per day, 6 days a week and a total duration of 2 weeks. On the 8th day of exposure, the female mice in both groups were superovulated and mated overnight. In the next morning, the female mice with a vaginal plug were identified as pregnant; at the time of implantation, the pregnant mice were killed and blastocysts obtained by flushing the uterus horns. The mean number of pregnant mice, blastocysts after flushing, and the DNA fragmentation index following TUNEL staining in both groups were compared using statistical methods (SPSS, t test, $p<0.05$).
Results: The results showed that the percent of pregnant mice was decreased in experimental group (50%) compared to control group (67.5%) but the difference was found to be in significant. In addition, the data also demonstrated that the mean number of blastocysts after flushing was decreased in EMF group compared with control group (5.5 ± 5.7 vs. 9 ± 4.8; $p \leq 0.03$). Although the mean number of blastomers were not significantly decreased in experimental group compared to control group, however, the DNA fragmentation index was significantly increased in EMF group comparing with control group (10.53% vs. 7.14%; $p<0.001$).
Conclusion: Our findings indicate that the EMF exposure in preimplantation stage has detrimental effects on female mouse fertility and embryo development by decreasing the number of blastocysts and increasing the blastocysts DNA fragmentation.

Keywords: Electromagnetic Field, Mouse, Blastocyst, Apoptosis

P-47: Investigation of Cell Death in Cumulus and Oocyte Complex (COCs) of Preovulatory Follicle Produced *In Vitro* after Exposure to Hydrostatic Pressure

Rashidi Z*, Azadbakht M, Amini A

Biology-Razi, Kermanshah, Iran
Email: rashidi.zahra@gmail.com

Objective: *In vitro* maturation of oocytes is a safe and effective treatment offered in some fertility centers for assisted reproduction. Cumulus cells play a critical role in oocyte maturation and fertilization. Whether the degree of cell death in the cumulus–oocyte complex (COCs) has an impact on oocyte development potential is unclear. Physical forces may to assure the incidence of cell death in (COCs). Hydrostatic pressure as a physical force is effective in reproductive system and there is an increase in intrafollicular pressure between 15-20 mmHg in the ovulating follicle during the late stage of the ovulatory process and COCs to expose intrafollicular pressure in preovulatory follicle. In this study, we examined the effects of hydrostatic pressure on the viability of COCs derived from preovulatory follicle produced in vitro.
Materials and Methods: Preantral follicles were isolated from 12-day-old female NMRI mice, each follicle cultured individually in microdrops 20 µl of MEM-α culture medium supplemented with 5% fetal bovine serum, 100 mIU/ml recombinant follicle stimulating hormone (Gonal-f), 10ng/ml recombinant epidermal growth factor, under detoxified mineral oil for 12 days. On day 12, follicles with diameter nearly 500 µm and good quality were induced using 7.5 IU/ml human chorionic gonadotropin for in vitro maturation. At the start of maturation period follicles divided into two groups; control and experiment. In experiment group follicles were transferred to pressure chamber and subjected to 20 mmHg hydrostatic pressure for 30 min and then follicles from two groups were cultured for 24-48 h. Viability of cumulus cells and oocyte were assessed with nuclear differential staining (propidium iodide & bisbenzimide) on 0 and 24 h after culture.
Results: Our results indicate that, viability of the cumulus cells were reduced in hydrostatic pressure treated follicles compared to control ($p<0.05$). Percentage of condensed and fragmented nuclear in cumulus cells were increased in hydrostatic pressure treated follicles compared to control ($p<0.05$). Hydrostatic pressure no effect on oocyte viability in 0 and 24 h after culture ($p<0.05$).
Conclusion: Hydrostatic pressure had the mild effect on cell death incidence in cumulus cells without any effect on oocyte. It be concluded that Hydrostatic pressure can be used to induce cell death cumulus–oocyte complex (COCs). It may improve releasing and mediating signals from cumulus cells to oocyte.

Keywords: IVM, Cumulus Oocyte Complex, Hydrostatic Pressure, Cell Death, Mouse

P-48: *In Vitro* Maturation of Oocytes Derived from Preovulatory Follicles Produced *In Vitro* After Exposure to Hydrostatic Pressure

Rashidi Z*, Azadbakht M, Amini A

Biology Razi, Kermanshah, Iran
Email: rashidi.zahr@gmail.com

Objective: *In vitro* maturation (IVM) of mammalian oocytes has become an efficient method to produce mature oocytes in order to use in assisted reproductive techniques. Induction of ovulation to obtain mature oocytes for IVF has become a routine procedure in many infertility clinics. Some women, however, rather fail to respond to the hormonal stimulation or are at risk of ovarian hyperstimulation. In vitro maturation of oocytes offers an alternative to obtain mature oocytes in these cases. Hydrostatic pressure as a physical force is effective in reproduction system and there is an increase in intrafollicular pressure between 15-20 mmHg in the ovulating follicle during the late stage of the ovulatory process. In this study, we examined the effect of hydrostatic pressure on in vitro maturation of oocyte derived from preovulatory follicle produced in vitro.
Material and Methods: Preantral follicles were isolated from 12-14 day-old female NMRI mice, each follicle cultured individually in microdrops of MEM-α culture medium supplemented with 5% FBS, 100 mIU/ml rFSH (Gonal-f), 10ng/ml recombinant Epidermal Growth Factor, under detoxified mineral oil for 12 days. On day 12, follicles with diameter ≥500 µm and good quality were induced using 7.5 IU/ml human chorionic gonadotropin for oocyte maturation. At the start of in vitro maturation follicles divided into two groups, control and experiment. In experiment group, follicles were subjected to 20 mmHg hydrostatic pressures in pressure chamber for 30 min. Then follicles from two groups were cultured for assessed in vitro maturation of oocyte.
Results: Our results showed that, after 24 h the percentage of metaphase (MII) oocyte increased in hydrostatic pressure treated follicles (16%) compared to control (9%)($p<0.05$). After 48 h the percentage of metaphase (MII) oocyte increased in hydrostatic pressure treated follicles (33%) compared to control (20%)($p<0.05$).
Conclusion :
Conclusion: It be concluded that hydrostatic pressure can be to improve oocyte in vitro maturation.

Keyword: *In Vitro* Maturation, Hydrostatic Pressure, Oocyte, Mouse

P-49: Maturation Arrest of Sperm after Subchronic Chemotherapy

Rezvanfar M*, Shojaei Sadee H, Abdollahi M

Laboratory of Toxicology, Faculty of Pharmacy, and Pharmaceutical Sciences Research Centre, Tehran University of Medical Sciences, Tehran, 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. In this context, we have studied the CP induced oxidative injury in rat sperm.
Materials and Methods: Animals were randomly divided into two groups comprised of 8 animals in each. Treatment groups were as follow: group 1 received distilled water by oral gavage daily and group 2 received CP (6 mg/kg/day) dissolved in distilled water by gavage. The groups were treated for 4 weeks. The protocol for this study, including doses and duration of treatment for CP, were all designed according to previous studies. At the end of the specified treatment, the samples of plasma, testes and epididymides were collected for the analysis of free radical toxic stress markers including cellular lipid peroxidation (LPO) and total antioxidant power (TAP) and histopathological study.
Results: The testes of CP-exposed rats showed a significant increase in lipid peroxidation, along with a significant decrease in total antioxidant power. Histopathological assessment of sperm concentration performed on cauda epididymis sections from CP-treated animals showed severe decrease of mature spermatozoa concentration in lumen. Histopathological examination of testis sections revealed inhibition of spermatogenesis and the preferential loss of maturing and elongated spermatids. qualitative examination of testicular sections revealed fewer mature luminal spermatozoa in comparison to the control. In CP-treated animals, there was seen disorganized germ cells epithelium in the most of seminiferous tubules along with degenerated and necrotic cells in some of seminiferus tubules. In some section of seminiferous tubules, large number of metaphasic cells in germ epithelium was observed.
Conclusion: Cyclophosphamide treatment at the dosage used caused testicular gametogenic disorders and arrest of sperm maturation via induction of oxidative stress.

Keywords: Cyclophosphamide, Testicular Toxicity, Spermatogenesis, Oxidative Stress

P-50: Tamoxifen Affects Early Oocyte Differentiation in Mice

Roshangar L*, Soleimani Rad J, Afsordeh K

Embryology Department, Tabriz University of Medical Sciences, Tabriz, Iran
Email: lroshangar@yahoo.com

Objective: In human, early oocyte differentiation occurs during fetal life. But in mice, it takes place in early postnatal period, providing a valuable model for investigating the effect of different factors on oocyte differentiation. It is thought that estrogen may have a role on early oocyte development and therefore the aim of the present study is to evaluate the effect of tamoxifen on early oocyte differentiation and follicular development in mice.
Materials and Methods: In this study 30 adult female and 15 adult male mice are used. Two female mice at their sterous cycle were housed in a cage for mating. Observation of vaginal plaque was considered as the first day of pregnancy and the mice on the 13th day of pregnancy received 100 micro gram tamoxifen as ip injection. After delivery, the 2, 3, 6 and 7 days old pups were sacrificed and their ovaries removed and fixed and prepared for light and electron microscopy. Ultrastructural morphology of differentiating oocytes were studied and the number of oocyte nests and diameter of primordial and primary follicles were determined.
Results: Microscopy showed that oocyte nests were formed on 2-3 day old pups and follicles were distinguished on 6 and 7 days. Morphometric studies revealed that the number and diameter of oocyte nests were significantly reduced in experimental group, in comparison to control group ($p < 0.001$). However, the number and diameter of primordial and primary follicles were similar in both groups. Electron microscopic studies revealed that in control group oocytes were separated from each other and were mainly in the form of primordial follicles. However, in the experimental group, they mainly were in the form of oocyte nests.
Conclusion: The results indicate that tamoxifen suppresses oocyte differentiation at early stages but does not affect the development of already differentiated follicles.

Keywords: Folliculogenesis, Mouse, Oocyte Nest, Tamoxifen

P-51: Effects of Subcutaneous Nicotine Administration on Rat Sperm Parameters

Sarve-Azad A[1]*, Ezzatabadipour M[1], Azizollahi S[2], Mirkahnooj Z[3], Nematollahi-Mahani S.N[1]

1. Department of Anatomy, Afzalipour School of Medicine, Kerman University of Medical Sciences, Kerman, Iran
2. School of Veterinary Science, Shahid Bahonar University, Kerman, Iran
3. Afzalipour School of Medicine, Kerman University of Medical Sciences, Kerman, Iran
Email: enshroud_man@yahoo.com

Objective: The effects of nicotine on sperm count, motility, and viability was evaluated in male rats and the results were compared to control.
Materials and Methods: Thirty healthy Spauge-Dawley male rats (average weight 160-210 g) were randomly divided to 3 groups (n=10). Animals received a subcutaneous injection of 0.02 mg/Kg nicotine for 50 days. A group of animals received the same volume of vehicle and served as control. An intact group received no intervention and served to evaluate probable age changes in the reproductive system and to consider any stress induced by the treatments. For Sperm analysis, Cauda epididymis and vas deference were placed in 5 ml α-MEM supplemented with 2% BSA and cut into several fragments to allow the spermatozoa come out from reproductive ducts. Samples were incubated for 15 min. at 37°C and the following experiments were carried out on sperm suspensions in various groups. Sperm quality was determined by three parameters; concentration, motility and viability. The viability was assessed by three methods; Eosin exclusion dye test, Hypo-osmotic swelling test and Propidium iodide exclusion test.
Results: A reduction in sperm concentration was observed in nicotine group ($p<0.01$) compared to control. Motility was also not significantly impaired in nicotine group compared to control group, but sperm viability, evaluated by either of the methods was non- significantly altered.
Conclusion: According to our study, it seems that nicotine has some toxic effects on male reproductive system and alters sperm parameters.

Keywords: Nicotine, Sperm, Rat

P-52: *In Vitro* Developmental Competence of Bovine Embryos After Biopsy at Different Embryonic Age and Stage

Shirazi A*, Borjian S, Nazari H, Ahmadi E, Heidari B, Bahiraee A

Research Institute of Animal Embryo Technology, Shahrekord, Iran
Email: borjian_sara@yahoo.com

Objective: The objective of the present study was to assess the in vitro development of bovine embryos biopsied at different days of precompacted morula stage.
Materials and Methods: Slaughterhouse - derived oocytes were matured in vitro, fertilized (Day 0) with frozen-thawed, Percol-separated spermatozoa and cultured on oviductal cell monolayer. In vitro fertilized embryos were subjected to cell biopsy on days 2, 3, and 4 post-insemination at 4 to 16-cell stages in a 100µl drop of Hepes-SOF + 4mg/ml BSA by aspiration (1-2 cells). Biopsied embryos cultured on oviductal cell monolayer for subsequent development.
Results: Biopsy on 16-cell stage embryos (Day 4) resulted in 94% of embryos proceeding to the blastocyst stage, which was higher than those which had been biopsied at Day 4 (64%; 8-cell stage, $p<0.01$), Day 2 (39%; 4-cell stage and 33%; 8-cell stage, $p<0.01$) and Day 3 (49%; 4-cell stage and 46%; 8-cell stage, $p<0.01$).
Conclusion: In conclusion biopsy at precompacted morula stage had no harmful effects on in vitro developmental potential of bovine embryos and that the 16-cell stage embryos at Day 4 were the best candidate for blastomere removal.

Keywords: Embryo Biopsy, Bovine, *In Vitro* Production

P-53: Ultrastructural Study of Endometrial Luminal Epithelium In Superovulated Mice Treated With Progesterone or Viagra

Soleimani Rad J*, Rashidi B, Roshangar L[2], Soleimani Rad S

1. Embryology Department, Tabriz University of Medical Sciences, Tabriz, Iran
2. Drug Applied Research Centery, Tabriz, Iran
Email: lroshangar@yahoo.com

Objective: Low implantation rate in ART implies the necessity for accelerating endometrial receptivity. Progesterone has commonly been used for this purpose. In the present study, the ultrastructural characteristics of endometrial luminal epithelium has been used to evaluate and compare the effect of progesterone and Viagra on endometrial receptivity in pre-implantation stage.
Materials and Methods: Forty adult female Balb-c mice were divided into one control and three experimental groups. In 3 experimental groups, the mice received 7.5 IU hMG and later hCG. Then every two female mice with one male mouse housed in one cage for mating. In one of the experimental group, 1mg progesterone/mouse and in the other 3mg/kg Viagra was administered in 24, 48, 72 hour intervals after hMG injection. Ninety six hours after hMG injection, the mice in all groups, were sacrificed and from those that their uterine tube contained blastocyst the uterine specimens were collected and prepared for electron microscopy (LEO 906).
Results: Microscopy showed that, in the control group, luminal epithelial cells were high columnar cells containing euchromatin nuclei, numerous microvilli, several mitochondria at apical cytoplasm and many subnuclear granules. In experimental groups, the cells by having short microvilli, several supranuclear granules and development of pinopodes were different than control group. However, in case of pinopodes; the group that received gonadotropin + progesterone was similar to control group, but the group that received gonadotropin and Viagra were similar to the group that had received only gonadotropin.
Conclusion: It is concluded that Viagra may promote some other aspects of endometrium which are considered as increased endometrial receptivity.

Keywords: Pinopodes, Viagra, Endometrial Receptivity

P-54: Intrauterine Insemination with Husband Semen: An Evaluation of Pregnancy Rate and Factors Affecting Outcome

Zademodarres Sh*, Oladi B, Saeedi Sh, Jahed F

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

Objective: The aim was to determine pregnancy rate following intrauterine insemination (IUI) and its associated factors.
Materials and Methods: A retrospective analysis of 350 IUI cycles with ovarian stimulation by clomiphene citrate and/or gonadotropins was performed.
Results: The overall pregnancy rate was 22% (77/350). Of the 77 pregnancies, 88.3% resulted in live birth, 7.8% in spontaneous abortion, 2.6% in blighted ovum and 1.3% were ectopic
Conclusion: Our results indicate that clomiphene citrate and/or gonadotropins IUI is a convenient and useful treatment option in women with younger age (<30 years) and fewer treatment cycles and fewer infertility duration (4 years).

Keywords: Clomiphene Citrate, Human Menopausal Gonadotropin, Infertility, Intrauterine Insemination, Pregnancy Rate

P-55: Early Pregnancy Loss Following Intracytoplasmic Sperm Injection

Zeinalzadeh M[1]*, Buzari Z.A[2], Yousefnia Pasha Y.R[1]

1. Fatemehzahra Infertility Research Center, Babol, Iran
2. Obstetrics and Gynecology, Babol University, Babol, Iran
Email: zeinalmahtab@yahoo.com

Objective: The aim of the current study was to investigate the some potential risk factors for EPL in ICSI pregnancies.
Materials and Methods: In this cross-sectional study during the period 2006 to 2008, we analyzed the 220 ICSI cycles that were carried out at the Centre for Reproductive , in Babol, Iran. Cases with oocyte donation cycles were excluded from this study. In ICSI cycles, all patients underwent long protocol. The risk factors for EPL including: maternal age, body mass index (BMI, kg/m2), polycystic ovary syndrome (PCOS) status, infertility aetiology and a quasi measurement of embryo quality were compared by $\chi 2$-test between two groups of patients, with (44 patients) and without (176 patients) EPL.
Results: Overall EPL was 20%. In EPL group, percent of patients >=35 years old were significantly higher than control ($p<0.05$). There were not significant difference between EPL and control about age, BMI and oocyte recovered ($p>0.05$). Percent of embryo transfer with good quality in the EPL group was significantly lower than control ($p<0.05$). In the EPL group, patient with male factor was significantly lower than control ($p<0.05$), but percent of female factor between two groups were not significant ($p>0.05$). There was no effect of PCOS.
Conclusion: Results shown that there was relation between EPL and maternal age, embryo quality and male factor infertility.

Keywords: Intracytoplasmic Sperm Injection, Early Pregnancy Loss, Risk Factors

Epidemiology and Ethics

P-56: The Effects of Group Cognitive - Behavior Therapy on Increas Marital Satisfaction in the Primary Infertile Women

Gharaei V

Hakimi Counseling Center ,Mashhad ,Iran .
Email: v_gharaie@yahoo.com

Objective: Infertility is a complex crisis in life,that leads to psychological and emotional stress in couples. The purpose of this study was the effects of group cognitive - behavior therapy on increas marital satisfaction in the primary infertile women.
Materials and Methods: : We studied 20 women with primary infertility who were undergoing IVF referring to Mashhad Treament Center.patients were randomly allocated into (10 experimental and 10 control) groups. First,they completed interviews and DAS questionnaires(Dyadic Adjustment Scale).Then,the intervention was conducted in experimental group,with one session per week for 4 months. The sessions included a therapy program comprised of modules to behaviorally optimize the chance of conception,improve sexual functioning and satisfaction,reduce thoughts of helplessness and improve marital communication skills.Reassessment occurred after completion of the intervention.The data analyzed through independent t-test with SPSS.
Results: The results revealed that there was a significant difference ($\alpha=0/01$) between two groups. Findings showed that patients who received group cognitive - behavior therapy reported more marital satisfaction than the patients who didn't received any interventions.
Conclusion: The group cognitive-behavior therapy showed a reduction in thoughts of helplessness and a decrease in marital distress. By the end of therapy participants reported unchanged sexual pleasure and satisfaction during the nonfertile period of the menstrual cycle.

Keywords: Group Cognitive - Behavior Therapy, Marital Satisfaction, Primary Infertile Women

P-57: Infertile Women's Responses to Assisted Reproductive Technologies in a Religious Context: Voices of a Multi-Faith Population

Latifnejad Roudsari R[1]*, Allan H.T[2], Smith P.A[2]

1. Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran
2. Health and Social Care, University of Surrey, Guildford, UK
Email: latifnejadr@mums.ac.ir

Objective: Scientific literature has identified religion as a factor which can affect the practice of reproductive technologies. This study explored how religious/ spiritual infertile women responded to ART using their religious teachings.
Materials and Methods: In a feminist grounded theory study 30 infertile women affiliated to different denominations of Christianity (Protestantism, Catholicism and Orthodoxy) and Islam (Shi'a and Sunni) were interviewed. Participants were recruited using theoretical sampling in one Iranian and two UK fertility clinics. Data were analyzed using Strauss and Corbin's mode of grounded theory.
Results: Three categories emerged embracing: exploring religious authorizations, faith-based decision-making and transcendent hope to attain a pregnancy. The majority of religious infertile women endeavored to explore religious scholars' views on ART. To make decision to do ART the mainstream of Muslims and Christians deemed IVF as a kind of advanced technology of reproduction which has no religious prohibition. Regarding gamete/ embryo donation, they expressed a wide variety of outlooks including opposition, agreement, and ambiguity. The majority of Sunni Muslims and Christians expressed their objection with gamete donation. In contrast, the majority of Shi'a Muslims, who were religiously allowed to use donor procedures, and a few number of Protestant, Catholic and Orthodox participants, despite not being religiously allowed, had no opposition with gamete donation. Some participants experienced ambiguity and uncertainty. Religious women had a transcendent hope to attain a successful pregnancy, which arose from their belief in God's blessing and miracles.
Conclusion: Health professionals should be aware of religious/ spiritual infertile women's tendency to use their belief system to make decision on ART, although a minority may not act upon religious authorizations for the reason of struggling with the desperation and heartbreak caused by infertility.

Keywords: Infertility, Religion, Spirituality, Assisted Reproductive Technologies, Feminist Grounded Theory

P-58: Ethical Considerations Around Sex Selection

Sabah Mozafari*, Reza Omani Samani

Department of Epidemiology and Reproductive Health, Royan Institute for Reproductive Biomedicine, Reproductive Biomedicine, ACECR, Tehran, Iran
Email: samani@royaninstitute.org

History reveals that human has been looking for identification and the ways for selection of offspring gender eagerly. Although, intensity to this issue has decreased by time; however, it is remained yet. Actually, determination of the kid sex was so intended that some people have made many efforts among them including referral to magicians or looking it near the physicians, using special methods like timing of coitus and consumption of different materials or drugs before pregnancy, or abortion of the fetus and even doing infanticide after birth in order to exerting sex selection depend up their different needs, cultures or religions. Nevertheless, only recently have medical technologies made it more possible to attempt sex selection of children before their conception of birth. These are included sperms flowcytometery and preimplantation genetic diagnosis (PGD).
It seems that sex selection is associated with several considerable aspects from which some are its advantages and others look like to be its disadvantages. In fact, whether overall or universal helpfulness of using these technologies in serving fertile or infertile parents to choose the sex of their children for either medical or nonmedical reasons is ethical or not, is more controversial in different countries for diverse reasons.
There is a universal consent around using of PGD for sex identification in parents with a high risk to birth the offspring with genetic disorders. Sex selection may also be used for balancing of the children gender in a family or may be used just because of the parents are intended to have a child with a special sex for any reason. Pro-sex selection communities claim that parents have rights and must to be free to select the sex of their children. However, do really we have right to select the characteristics of our child because we have right to reproduce?
"All rights must to be reserved unless it could cause damage(s)"; Robertson said. Actually, sex selection may make some useful or harmful consequences for offspring, parents or society. For example it has not really cleared that these medical technologies are completely harmless. The child with selected sex may underlie more unreasonable expectations and pressures by parents. Child may be behaved differently and understanding of his/her fact might be caused him/her to compare his/herself with others or might check himself obsessively and it might lower his/her self steam. The parents might treat with prejudice between their children. Even this is probable that the sex selection might be down unsuccessfully and the fetus or offspring with unwanted sex might be aborted or behaved poorly. Some families might be disintegrated without sex selection or even in some country in which polygamy is legal it could lead parents to break up or remarriage in order to birth child with desirable sex. In some countries women are obligated to bear so many difficulties either following numerous pregnancies

to birth child with desirable sex or even to safeguard the family foundation. Sex selection could help people to minimize family population especially in families with low incoming.
In countries with unequal sex rights, liberality in sex selection would worsen or in part reversed the slow advances in adjusting gender prejudice and would reinforce incorrect customs or wrongful traditions. Universal sex selection especially in some developing countries might threat the societies for inequality in population sex ratio which in turn could lead to difficulty to mate or immorality, violations or encroachments to the least sex. Other necessary considerations are gradual lead toward baby planning which might start via sex selection. Moreover, for PGD it is needed to generate numerous embryos which their extra creations are immoral in almost societies. Hence, in many countries this is still remained uncertain that whether sex selection is allowed to carry out for nonmedical reasons or if performances of IVF and PGD are ethical to serve for fertile parents too?
In conclusion, we suppose that family balancing via sex selection for nonmedical reasons and just for second or subsequent children might be helpful if and only if we could prove that there is no any tendency to a specific sex in the given society and sex selection in cases with medical reasons would be preferred at all. For sex selection in fertile parents who don't need IVF it is better to use prefertiliztion methods in order to prevent extra embryos production and also government must to regard taxes for it with the aim of minimizing treasury consumptions for nonmedical cases.

P-59: Anonymity in Gamete and Embryo Donation

Mozafari M[1]*, Omani Samani R[2], Oraei A[2]

1. Tehran University, Tehran, Iran
2. Department of Epidemiology and Reproductive Health, Royan Institute for Reproductive Biomedicine, Reproductive Biomedicine, ACECR, Tehran, Iran
Email: mostafamozafari2004@yahoo.com

Objective: One of the most controversial issues in donation protocols is anonymity. This is mostly refers to disclosure the identity of the donor to recipient or to the resulting child. Some countries have positive and some have negative position toward anonymity. For example HEFA in UK have passed a rule that at 18th year of child's age, the donor identity and address must be given to the child and also it is considered as a right for him to contact or see his genetical parents. On the contrary, some other countries like Russia, Belgium, Spain, France and Zech republic have accepted the complete anonymity.
Materials and Methods: Still, in Iran, there is not any law or legislation in this regard, but, as mentioned in Article no: 167 of Iran Constitution, in the cases that there is no law, people can rely on decrees of clergy leaders "Ayatollahs".
Results: According on our investigation among decrees, there are two groups of clergy leaders, the first are against anonymity specially for child, and the second are in agree with it. In a society like Iran in which, donation children and parents rights are not well defined, and many of these rights and relations are directly connected to the lineage, donation without anonymity may put the families and children in danger.
Conclusion: The advantages and disadvantages of anonymity in Iranian society are discussed in this paper along with basis of the clergies' ideas about it.

Keywords: Donation, Islam, Law, Anonymity

P-60: A Study about Educational Needs of Teenage Girls Toward Reproductive Health and Determination of a Proper Strategies to Provide It in Qazvin

Olfati F[1]*, Aligholi S[2]

Midwifery Faculty, Qazvin University, Qazvin, Iran
Email: papoy6olfati@yahoo.com

Objective: Adolescence is one of third critical period of life with a variety of developmental changes. Adolescence Reproductive Heath (R.H) has key role in providing the their health. Objective: The aims of this paper are to studying the educational needs of adolescent toward Reproductive Health and determination effective strategies to educate them.
Materials and Methods: This Cross sectional study conducted on 300 teenagers girl in 6 schools of Qazvin (randomly selected) in 2005.Data was analyzd by Chi-square tast and T.test .
Results: Average scale of knowledge about exercise and nutrition were 29, anatomy 11, reproductive physiology 13, menstruation 36, puberty heath 35, breast cancer 15, AIDS & STDs 60, family planning methods 27.
Conclusion: In order to results of this study, knowledge of is quite weak. Therefor R.H education via schools midwifes, mothers, teachers, friends, etc in adolescent age group is considering.

Keywords: Reproductive Health, Educational and Needs, Adolescent

P-61: A Survey on the Knowledge and Attitude of the Male Employees of the Health Center Towards Vasectomy

Seyyed Aghamiri Z.B*, Hosseini N.S, Kakaei F

Salamat Deputy, Tehran University, Tehran, Iran
Email: n.h1386@yahoo.com

Objective: Vasectomy is a safe and effective way to permanently prevent pregnancy. It is a small procedure that can be carried out in less than 20 minutes. Preparing the patient for the operation and giving him

local analgesia takes less than a few minutes. In the procedure, one or two small cuts are made on the scrotum, which enables the doctor to block the vas deferens. The cuts are then being closed. This operation has very few side effects, has nothing to do with the natural process of sexual activities, and no dangers are known to be included.
Materials and Methods: A knowledge-based questionnaire of 14 questions, and an attitude-based one of 14 more were prepared and been given to 102 randomly chosen male employees of The Personal Hygiene Center in the city of Sari. After collecting the questioners, the answers were analyzed by SPSS, ver. 11.0.
Results: The average age of the sample participants was 37.8 ± 5.2. 73% of the samples were unmarried, and 4.76% of them were childless. The rest had between 1 to 5 children. 12.8% had a good deal of information (Scores: 31-36), 48.67% had average amount of information (Scores: 27-30), and 38.61% had little information (Score<27). 2.89% have a positive attitude, 49.8% ranged from positive to indifference, and 48.1% had a negative attitude towards the matter.
Conclusion: According to the results, direct and indirect instructional and educational plans are needed in order to promote the knowledge and attitude of Health System employees, as the representatives of family planning programs in the society.

Keywords: Knowledge, Attitude, Vasectomy

Female Infertility

P-62: The Relationship between Preeclampsia and Polycystic Ovarian Syndrome is not Affected by Obesity

Aali B.S*, Mahdi S.A

Kerman University of Medical Sciences, Kerman, Iran
Emial: shahnaz.aali@gmail.com

Objective: Polycystic ovarian syndrome (PCOS) is considered as a predisposing factor for preeclampsia. There is debate regarding the role of obesity against PCOS per se as an implicating factor in preeclampsia. The present study was carried out to assess the role of PCOS without obesity in the development of preeclampsia.
Materials and Methods: In a case- control study 75 patients with preeclamsia and 225 age and gravidity matched normal pregnant women were studied for previous sign and symptoms of PCOS. Chronic underlying disease and endocrine disorders were excluded and both groups were classified as lean to normal when BMI was less than 25 and overweight to obese with BMI>25. BMI was calculated based on the weight before 10th week of pregnancy and current height. The groups were compared based on their positive history of PCOS and pvalues<0.05 were considered as significant.
Results: Mean BMI was greater in preeclamptic patients than the contol group. History of previous PCOS was found in a significantly higher number of preeclamptic patients. Further, preeclampsia showed a significant relationship with PCOS in lean to normal weight patients.
Conclusion: Preeclampsia is more prevalent in women with PCOS and this relationship is independent from obesity.

Keywords: Polycystic Ovary Syndrome, Preeclampsia, BMI

P-63: Secretion of VEGF Following three Dimensional Culture of Human Endometrial Tissue: an *In Vitro* Endometriosis Model

Ai J[1]*, Esfandiari N[2], Casper R.F[2]

1. Tissue Engineering Department of Medical University of Tehran, Tehran, Iran
2. Division of Reproductive Sciences, Department of Obstetrics and Gynecology, Samuel Lunenfeld Research Institute, Toronto, Ontario, Canada
Email: jafar_ay2000@yahoo.com

Objective: Endometriosis is the presence of endometrial glandular and stromal cells outside of the uterine cavity. This disease is found in about 10% of women of the reproductive age and in up to 50% of women with infertility. Surgery continues as the first-line treatment to eradicate endometriotic lesions but recurrence of the condition occurs in up to 47% of women.VEGF is an effective factor in establishment of endometriosis. The aim of the present study is to determine VEGF levels in human culture of endometrial fragment
Materials and Methods: Endometrial biopsy in Premenopausal patient's women referred to T.C.A.R.T (Toronto Centre for Advanced Reproductive Technology) for infertility treatment such as uterine myomas or ovarian cyst. Endometrial samples were collected from a total of ten normal ovulating women on cycle days 19-24. The biopsies were obtained from the fundal region of the uterine cavity. Ten tissue fragments were cultured by three dimensional methods for each patient. Supernatant fluid sample was collected from endometrial samples which were cultured in a three-dimensional fibrin matrix.
Results: Level of VEGF in Supernatant fluid of endometrial samples was placed in a three-dimensional fibrin matrix culture system were determined. These data showed Cell proliferation was observed in 91% of the wells. Angiogenesis was observed in 51 wells that showed cell proliferation (56%). The level of PRL in the supernatant fluid of wells that showed angiogenesis were increased (P<0.05) compare to supernatant fluid of wells that didn't show angiogenesis.
Conclusion: VEGF probably play an important role in promoting neovascularization and cell proliferation in establishment endometriosis. VEGF is involved in the regeneration of the endometrium and may be in establishment of endometriosis.

Keywords: Endometriosis, Endometrium, *In Vitro*, Three Dimensional Tissue Culture, Angiogenesis, VEGF

P-64: Effects of Pentoxifylline and Vitamin E on Pregnancy Rate and Endometrial Thickness in Infertile Women Treated by ZIFT

Aleyasin A*, Agahosseini, Mohseni M, Mahdavi A

Tehran University of Medical Sciences, Tehran, Iran
Email: atossa_mahdavi@yahoo.com

Objective: To determine whether combined pentoxifylline (PTX) and tocopherol (vitamin E) treatment can improve clinical pregnancy and endometrial thickness.
Materials and Methods: One hundred twelve infertile women undergoing standardized controlled ovarian hyper stimulation for ICSI- ZIFT entered this randomized clinical trial. Patients were randomized to two groups of combined drug therapy or none. Pentoxifylline 400 mg/BD plus vitamin E 400 mg/BD were administered to the intervention group for two cycles before starting ICSI/ZIFT cycle until detecting positive beta-hCG or the cycle was cancelled. The other group did not receive combination drug therapy. Main outcome measures were clinical pregnancy and endometrial thickness.
Results: The clinical pregnancy was higher in the intervension group in comparison to the other group (57.14% vs 39.29%, p=0.01). However, there was no difference in the mean endometrial thickness, number of retrieved oocytes, the number of metaphase 2 oocytes and grade of them in both groups.
Conclusion: This study showed that Pentoxifylline plus vitamin E could improve the ZIFT outcome in infertile couples. Local effects and antioxidative characteristics of these drugs may be the cause of better results.

Keywords: Endometrium, Pentoxifylline, Tocopherol, ZIFT

P-65: GnRH Antagonist/Letrozole Versus Microdose GnRH Agonist Flare Protocol in Poor Responders Undergoing *In Vitro* Fertilization

Davar R[1]*, Oskouian H[1], Ahmadi S[1], Oskouian L[2]

1. Research and Clinical Center for Infertility, Yazd, Iran
2. Mashad University of Medical Science, Mashad, Iran
Email: homaoskouian@gmail.com

Objective: To evaluate and compare the efficacy of a microdose GnRH agonist flare (MF) and a GnRH antagonist/letrozole (A/L) protocols in poor responders undergoing in vitro fertilization (IVF).
Materials and Methods: Ninety-four poor responder patients were randomized to an ovarian stimulation protocol with either a MF or a letrozole and high dose FSH/hMG and flexible GnRH antagonist protocol.
Results: There were no significant difference in mean age, body mass index (BMI), basal serum FSH and estradiol (E2) levels, duration of infertility, distribution of etiology of infertility and the number of previous failed IVF cycles. The days of stimulation, mean gonadotropin dose, the number of mature follicles and oocytes retrieved and metaphase II oocytes retrieved, serum E2 level on the day of hCG administration and the percentage of top and good quality embryos were significantly higher in the MF group. The endometrial thickness, the fertilization rate and the number of embryos transferred were similar in both groups. The implantation and clinical pregnancy rates were higher in the MF group and the total cancellation rate were higher in the A/L group, but these findings were not statistically significant.
Conclusion: The addition of letrozole to GnRH antagonist for poor responders does not improve outcome of assisted reproductive technology (ART) cycles. The MF protocol remains the most appropriate protocol in poor responders.

Keywords: Poor Responders, GnRH Antagonist, GnRH Agonist, Letrozole, In Vitro Fertilization, Controlled Ovarian Hyperstimulation

P-66: Evaluation of the Causes and Therapeutic Results of the Infertile Patients Undergo Laparoscopy

Erfanian Ahmadpoor M*, Mansoori Torshizi M

ART, Novin IVF Center, Mshhad, Iran
Email: mahmood_erfanian@yahoo.com

Objectives: Laparoscopy is one of the diagnostic and therapeutic methods in infertility. With this method, the causes of infertility (including pelvic, uterus and ovarian factors) that have not indicated in primary evaluations can be diagnosed and treated.
Materials and Methods: In a cohort study on infertile female patients reffering to NOVIN clinic of gynecology and infertility , after primary clinical and paraclinical evaluations of infertility and using of medical treatment, 140 patients candidated for laparoscopy, selected and undergo the procedure. Laparoscopic results evaluated in patients with primary and secondary infertility. Then, the patients were treated according to the laparoscopic results and were followed within one year for evaluation of the fertility outcomes (pregnancy rate and miscarriage).
Results: 74% of the patients had primary infertility and 26% had secondary infertility. The most common cause of primary infertility was PCOS and the most common cause of secondary infertility was tubal factor. The successful rate of pregnancy in patients with endometriosis were 58.1%, tubal factor 44.1%, PCOS 66.7%, uterus anomaly 40%, PCOS with endometriosis 71.4% and PCOS with tubal factor 22.2%. The rate of miscarriage in patients with endometriosis were 6.4% , tubal factor 8.8% , PCOS 2.5% and PCOS with tubal factor

11.1%. in patients with uterus anomaly and PCOS with endometriosis , no miscarriage were observed.
Conclusion: According to the results of this study, laparoscopy is a safe method in diagnosis the uterus, pelvic and ovarian factors and can improve the fertility outcomes in the infertile patients.

Keywords: Infertility, Laparoscopy, PCOS, Endometriosis

P-67: Endometrial Biopsy May Increase Endometrial Thickness and Implantation Rate in ART Cycles

Farimani M*, Dibavand N, Rabiee S, Bagheri M, Amiri I, Yavangi M, Bahmanzadeh M

IVF Center, Hamedan University of Medical Sciences, Hamedan, Iran
Email: dr_farimani@yahoo.com

Objective: In recent years, much attention focused on improvement of implantation rate in ART cycles. Human in vitro fertilization and embryo transfer (IVF/ET) is accompanied by a low implantation rate even after a very successful IVF procedure. The mechanism of human embryo implantation is poorly understood and the causes of many implantation failures are not known. It is evident that molecular interactions at the embryo maternal interface at the time of implantation are crucial and inflammatory cytokines and growth factors are possibly involved in this process. The aim of our study was to analyze the effectiveness of endometrial biopsy, in increasing the rate of implantation and pregnancy in ART cycles.
Materials and Methods: In prospective clinical trail study in Hamedan IVF centre sixty patients who candidate for IVF/ICSI cycle were divided in two equal groups, in case group (no=30) A single endometrial biopsy was performed using a Novak catheter on luteal phase of the cycle prior to ART cycles. Data are shown as mean-SD and comparison of variables were done using SPSS, t-test and chi-squre.
Results: The comparison of characteristics of the study patients are shown in table 1.
Conclusion: These data suggest that endometrial biopsy significantly increases the endometrial thickness. Implantation and pregnancy rates in ART cycles increased when endometrial thickness increased. Several mechanisms are thought to be involved in improving implantation including secretion of inflammatory cytokines and growth factors. Study of a larger group of patients is needed to confirm.

Keywords: Endometrial Biopsy, Implantation, ART

P-68: Practice of Female Population Towards Breast Cancer in Shiraz City

Fereydouni B[1]*, Fereydouni Sh[2], Naderi S[1]

1. Biology Departmeny, Shiraz University, Shiraz, Iran
2. Education Psychology, Gachsaran University, Gachsaran, Iran
Email: b.fereidooni@gmail.com

Objective: The study was aimed to assess the awareness about breast self examination (BSE) and coverage of breast cancer screening investigations in Iran.
Materials and Methods: Study was carried out in Shiraz city, 300 adult females randomly were selected. Data was collected with questionnaire and analyzes with SPSS.
Results: The average age of participants was 34 years, majority of them 92% were married .Only 36% of the females knew how to do a BSE and practiced it. The main reason for not doing BSE was lack of knowledge about it (51%).20% of participants thought that they don't need to do BSE and 12% said that they don't have a breast complaint so it was useless to do examination. only 15% have had abreast problem .An even less number 10% had a breast investigation(Mammography).
Conclusion: Despite wide agreements over the need for early detection of breast cancer the knowledge and practice for BSE is very low.

Keywords: Breast Cancer, BSE, Shiraz City, Awareness.

P-69: Compairing Effect of Gabapentin and Brofen on Dysmenorrhea in Infertile Patients, A Pprospective Randomized Study

Fotouhi E*, Yasini Sh, Razavi K

Afshar Hospital, Stem Cell Department, Yazd University, Yazd, Iran
Email: dr_ehsanfotouhi@yahoo.com

Objective: Dysmenorrhea is a common phenomenon before periodic cycles.medical treatment with NSAIDs may be controled it very weak.but the usage of NSAIDs have some limitation and side-effects.One of the methods of decrease pain is prophylaxy treatment.the purpose of this study was compairing effect of Gabapentin as a dopamin-agonist and Brofen as a NSAIDs for prophylaxy to decrease dysmenorrhea.
Materials and Methods: In doube blind randomized study of 100 infertile patients with severe dysmenorrhea, for 3-5 days before starting the pain of periods 50 were randomized to treatment with Gabapentin and 50 to treatment with Brofen.Levels of pain and need to analgesic were measured before and after periods in two groups. Baseline clinical and demographic characteristics were measured.
Results: The patients consisted of 100 infertile females, with a mean age of 30 ± 9 years. There were significant differences in levels of pain (3.2 ± 0.33 vs 1.5 ± 0.4 mg/dL, p=0.043) concentrations, or need to analgesic (52 ± 12.8 vs 52 ± 12.8 mL/min, p=0.039) in Gabapentin

treatment group. Member of patients who was refered to clinical office was significantly longer in the Brofen group than in the treated group with Gabapentin(3.9 ± 1.5 vs 2.6 ± 0.7 days, p<0.001).
Conclusion: Member of patients who was refered to clinical office for control of dysmenorrhea was significantly longer in the Brofen group than in the treated group with Gabapentin (3.9 ± 1.5 vs 2.6 ± 0.7 days, p<0.001).

Keywords: Dysmenorhhea, Infertile, Gabapentin

P-70: The Immediate Medical Therapy after Laparoscopic Ovarian Diathermy

Rasekh Jahromi Athar*

Jahrom University of Medical Sciences, Jahrom, Iran
Email: Drrasekh@yahoo.com

Objective: PCOS is the most common endocrinopathic and reproductive disorders in women. The pathogenesis of PCOS is still controversial. There has long been an association of abnormal gonadotropin secretion with this syndrome. Hyperandrogenemia is principally ovarian in origin although the adrenal gland may contribute. During reproductive age, PCOS is associated with relevant reproductive morbidity including menstrual irregularity, anovulation, infertility, increased pregnancy loss, and complications of pregnancy. The goal of this research, influential immediate medical therapy after LOD.
Materials and Methods: This prospective clinical research evaluates the multiple influential factors on LOD outcomes, in 177 infertile Pcos ladies, during 4 years. 77.6% was primary infertility, 20.4% secondry, 2% unknown cause. Women's average age is 27 and her husband 33. After evaluation of semen analysis, and ruled out other causes of hyperandrogenemia, Metformin and Clomiphene were prescribed for 3 months, if no response, HSG was performed for detection of tubal patency, then gonadotropins were added to previous drugs. IUI was tried in 21.7% of patients. TVS for follicular monitoring was performed. The data were analyzed with SPSS software. LOD was end goal to reduce the amount of androgen-producing tissue, and was continuing medical therapy immediately.
Results: No accessible 44 patients, therefore from 133 pcos ladies, 76 conception(55.6%), 49.7% term pregnancy, 5.9% abortion, due to LOD operation, and immediate medical therapy
Conclusion: Medical prescription, immediately after LOD was suggested, because prevention of recurrent increased androgenemia and gonadoropins, therefore, fertility chance will be improved but, this research will be necessitated for more study.

Keywords: PCOS, LOD, Infertility, Hyperandrogenemia

P-71: Local Injury to the Endometrium on the Day of Oocyte Retrieval Has a Negative Impact on Implantation in Assisted Reproductive Cycles: A Randomized Controlled Trial

Karimzade M.A*, Oskouian H, Ahmadi S

Research and Clinical Center for Infertility, Yazd, Iran
Email: homaoskouian@gmail.com

Objective: To evaluate the effect of local injury to the endometrium on the day of oocyte retrieval on implantation and pregnancy rates in assisted reproductive cycles.
Materials and Methods: In a prospective controlled trial, a total of 156 patients, < 38 years old, in their first IVF cycle were randomized. In 77 patients two small endometrial samples from anterior and posterior walls of uterus were obtained with a Novak curet on the day of oocyte retrieval and in 79 patients no intervention was performed
Results: The experimental and control patients were matched regarding women´s age, body mass index, basal FSH, duration and etiology of infertility, treatment protocol, number of retrieved oocyte, endometrial thickness, percentage of intracytoplasmic sperm injection performance, fertilization rate, the percentage of patients with good- and top-quality embryos and the number of embryos transferred. The implantation rate (7.9% vs. 22.9%), clinical (12.3% vs. 32.9%; odds ratio = 0.25; 95% confidence interval = 0.12-0.66; p < 0.05) and ongoing pregnancy (9.6% vs. 29.1%; odds ratio = 0.25; 95% confidence interval = 0.10-0.64; p < 0.05) rates were significantly lower in experimental group, compared with 79 controls.
Conclusion: According to the results of this study, local injury to the endometrium on the day of oocyte retrieval disrupts the receptive endometrium and has a negative impact on implantation and IVF outcomes.

Keywords: Endometrial Receptivity, Local Injury, Implantation, IVF Outcome

P-72: Metabolic and Endocrine Effect of Metformin and Metformin Plus Cyclic Dydrogestrel in Women with Polycystic Ovarian Syndrome

Parisa Moghtadaei*, Fariba Sardari

Obstetric and Gynecology, Tehran, Iran
Emal: drmoghtadaei@gmail.com

Objective: To evaluate the metabolic and endocine effect of dydrogestrel therapy with or without metformin in women with PCOD.
Materials and Methods: In this prospective study of women with PCOD, 80 women received metformin 1000 mg , however, 80 cases received metformin 1000 mg plus dydrogestrel 10 mg daily. Body mass index, hormonal and lipid profiles , homocysteine blood level,

and insulin resistancy were recorded at baseline, 3, and 6 months.
Results: Total cholestrol levels decreased in metformin plus dydrogestrel group (p=0.002) compared with not significant change in metformin in group.In contrast with metformin plus dydrogestrel , significant increased level in homocysteine was seen in mwtformin group(p=0.002).
Conclusion: There were no adverse effects of cyclic dydrogestrel plus metformin therapy on metabolic parameters in women with PCOD over a 6-months treatment period.

Keywords: Dydrogestrel, Metformin, Polycystic Ovarian Syndrome

P-73: Infertility and Polycystic Ovarian Syndrome: A Study of Association between Body Mass Index and Intrafamily Marriages

Haq Nawaz Fauzia*, Rizvi Javed

Aga Khan University, Karachi, Pakistan
Email: fauzia.nawaz@aku.edu

Objective: To determine the relationship of different clinical, biochemical parameters and hormonal assay with the BMI of women who are known to have PCOS, and to compare these demographic features with intrafamily marriages.
Materials and Methods: From January 2005 until December 2006, patients attending the infertility clinic at Aga Khan University Hospital, Karachi, were evaluated for their clinical features. Couples were divided into 2 groups: group A had a history of first-degree intrafamily marriages, and group B had none. Complete biochemical evaluation was performed by day-2 serum FSH, LH, prolactin, testosterone and fasting serum insulin levels.The results were recorded on a data collection form. Ultrasonic evaluation was performed with transvaginal ultrasound to check the morphological appearance of the ovaries. A modified glucose tolerance test with 75 g glucose was performed and the results were recorded as normal, impaired and abnormal. Insulin resistance was calculated using the HOMA index method.
Results: During this period 203 patients were evaluated for demographic and biochemical features of PCOS. The prevalence of obesity was 70% with 59.3% women found to have hyperinsulinemia while 52.3% of patients had insulin resistance according to the HOMA index method. Univariate and multivariate analyses were used to compare the 2 groups. A linear relationship between oligomenorrhea, family history of diabetes, tonic LH, high fasting serum insulin levels, insulin resistance and an abnormal glucose tolerance test was revealed, keeping intrafamily marriage and BMI as dependent variables. In this population 48% of couples were in first-degree intrafamily marriages, suggesting the possibility of a high genetic predisposition for abnormal metabolic features beside ethnic predisposition.
Conclusion: A linear relationship of high BMI and family marriages has been seen with insulin resistance, oligomenorrhea and impaired glycemic control. The number of obese women and the high rate of intrafamily marriages make our population genetically susceptible to metabolic complications.

Keywords: Polycystic Ovary Syndrome, Obesity, Body Mass Index, Infertility, Insulin Resistance

P-74: Breast Cancer and History of Infertility

Olfat Bakhsh, Mehrdad N, Sajadian A*

Iranian Center for Breast Cancer (ICBC) ,Tehran ,Iran .
E-mail: assajadi@yahoo.com

Objective: Infertility has been recognized by the WHO as a problem affecting between 15% and 20% of couples in developed countries. Several cohort studies of infertile women have reported an incidence of breast cancer similar to that of the general population. Breast cancer is associated with several risk factors such as infertility, Hormone therapy, late age at menopause, early age at menarche and late age at first full term pregnancy. Most of these risk factors coexist in infertile patients and some studies suggested that the different infertility causes can be involved in breast cancer risk development.
Materials and Methods: In a cross- sectional designed study, we assessed reproductive history for 665 breast cancer patients who treated in between January. 1997 and March.2009, Iranian Center for Breast Cancer (ICBC), Tehran, Iran. We retrieved demographic and reproductive characteristics (such as, age of menarche, history of hormone therapy, age at full term pregnancy, and menopausal status from the medical records of ICBC.
Results: Among 665 breast cancer patients with reproductive history, 24 (3.7%) had history of infertility. The mean age at the time of diagnosis was 46.4 years (SD = 11.3), most of them were married (N=645) (88%) and educated up to high school (67%). There were significant differences between early age of menarche ($p<0.02$) , hormone therapy ($p<0.0001$) and late age at full term pregnancy (22 and over) ($p<0.001$) with infertility treatment. There was no significant difference between menopausal statues with infertility treatment.
Conclusion: Despite many studies about infertility treatment and breast cancer, there aren't any available confirmed results about effects of infertility treatment on breast cancer risk. The study results suggested that hormone therapy may be important risk factor for breast cancer. So, it seems that further studies are needed about relationship between hormone therapy for infertility and breast cancer risk.

Keywords: Infertility, Breast Cancer, Hormone Therapy, Iran

P-75: Pregnancy Outcomes in Women Over 35 Years Old

Sekhavat L*

Obstetric and Gynecology, Yazd University, Yazd, Iran
Email: sekhavat@ssu.ac.ir

Objective: This study was performed to compare the outcomes of pregnancies in women aged 35 years and older with those of 20 to 30 year old women.
Materials and Methods: We reviewed the delivery records of 148 mothers aged 35 years and above (case group) who delivered since May 2006 to May 2008 and compared them with 240 younger ones (20-30 year old, control group). Multi-fetal pregnancies, hypertensive women and diabetic women were excluded. Data was analyzed by SPSS and $p < 0.05$ was significant.
Results: The mean age for the older group was 37.26 ± 3.62 years and that for the control group was 26.43 ± 4.37 years ($p > 0.05$). The mean duration of gestation was significantly shorter in oldergroup than control group (34. 54 ± 4.37 vs 37.12 ± 2.21, $p<0.05$). Preterm delivery was observed more frequently in the case group compared with controls (22% vs 9%, $p<0.05$). The incidences of preeclampsia, placenta abruption andcesarean delivery were significantly higher in the older group than control group ($p<0.05$). The mean birth weight was 2799 ± 541 g for the older group and 2,695 ± 647g for the control group ($p>0.05$). 5-minute Apgar scores of neonates have not significantly different between two groups.
Conclusion: Older pregnant women had increased risk of maternal complications but neonatal outcomes were similar to younger age group.

Keywords: Older Maternal Age, Pregnancy Outcomes, Maternal Complications

P-76: Review of Obesity and Reproductive in Men and Women

Yazdanpanahi Z*, Nematolahi A.N, Forouhary S.F

Nursing and Midwifery College, Shiraz University, Shiraz, Iran
Email: yazdanz@sums.ac.ir

Objective: To examine the association between body weight and measures of male reproductive potential
Materials and Methods: We review the association between BMI and Reproductive potential.
Results: In both sexes, obesity, may impair fertility. Some studies showed corroborate earlier findings that overweight and obese men have a markedly changed sex hormone profile in serum, and reduction of semen quality. In massively obese individuals, reduced spermatogenesis associated with severe hypo testosteronemia may favour infertility. Moreover, the frequency of erectile dysfunction increases with increasing body mass index .Furthermore there is a high prevalence of obese women in the infertile population ,and numerous studies have highlighted the link between obesity and infertility. in women, early onset of obesity favours the development of menses irregularities, chronic oligoanovulation and infertility in the adult age. Obesity in women can also increase risk of miscarriages and impair the outcomes of assisted reproductive technologies and pregnancy, when the body mass index exceeds 30 kg/m.
Conclusion: Fertility can be negatively affected by obesity. Weight reduction program with Change in life style and exercise should be an essential component of infertility management

Keywords: BMI, Reproductive Health, Obesity

Genetics

P-77: Study of Polymorphism in Intron 4 of the Endothelial Nitric Oxide Synthase Gene in Relation to Susceptibility to Miscarriage

Abbasi F[3]*, Sanati M.H[2], Habibi R[1], Chaparzadeh N[3], Gourabi H[1]

1. Department of Genetics, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. National Institute for Genetic Engineering and Biotechnology, Tehran, Iran
3. Tarbiat Moalem of Azarbayejan University, Tabriz, Iran
Email: rhabibi_ir@yahoo.com

Objective: Recent studies have indicated that genetic polymorphisms of the specific genes is one of the causes of miscarriage. In the other hand it is observed that nitric oxide (NO), as a signaling molecule, is participated in primary events of pregnancy such as implantation of blastocyst, differentiation of trophoblast, trophoblast invasion, and it enhances blood supply through the maternal arteries to the placenta. Thus it has a role in implantation and maintenance of pregnancy. NO is produced by endothelial nitric oxide synthase that is expressed in placenta, whereas this enzyme and its production play a key role in early stages of gestation, we investigated relationship between polymorphism of this gene and complication of pregnancy.
Materials and Methods: In this study we used the 37 women who had three or more constitutive miscarriage with unknown reason in first trimester of their pregnancy in case group. Control group is included 101 women with normal pregnancy. Both group were analyzed for the VNTR polymorphism in intron4 of endothelial nitric oxide synthase gene by polymerase chain reaction.
Results: In patient group genotype frequency of AB is 29.7 %, BB is 64.8 % and AA is 5.5 %. In control group, genotype frequency is 73.2 % for BB, 24.7 % for AB and 1.9 % for AA genotype.
Conclusion: The result shows that frequency of AA genotype in the patient group is more than control. Further studies with larger samples need to be done to con-

firm these findings.

Keywords: Pregnancy, Miscarriage, Nitric Oxide, Polymorphism

P-78: Prenatal Diagnosis of Chromosomal Abnormalities following Amniocentesis in a Maternity Hospital in Tehran

Behjati F[1, 2*], Bagherizadeh I[1], Shafeghati Y[1, 2], Hadipour Z[1], Hadipour F[1], Saremi A[3]

1. Medical Genetics Departemtn, Sarem Women's Hospital, Tehran, Iran
2. Genetics Research Center, USWR ,Tehran, Iran
3. Obstetric Gynecology Departemtn, Sarem Women's Hospital, Tehran, Iran
Email: f_behjati@uswr.ac.ir

Objective: To investigate the chromosome abnormalities in at risk pregnant women using fetal cells obtained from amniotic fluid at second trimester
Materials and Methods: A total number of 502 amniotic fluid samples were referred for chromosomal investigation to the Cytogenetics unit of Sarem Women's hospital for the past two years. Referral reasons included advanced maternal age (38%), abnormal maternal serum screening test (37%), previous abnormal child (10%), recurrent miscarriages (8%), abnormal sonography (2%), one parent with translocation (1.6%), and other reasons (3.4%). Three cultures were set up for each sample using amniomax/Ham's F10 media. The karyotype was carried out using standard GTG banding technique on the prepared chromosme spreads. A minimum of 20 cells were examined.
Results: Culturing success rate was 99.6%. Karyotype results are as follows: 47% of samples were male and 53% appeared as female karyotype. 27 out of 502 (5.4%) had abnormal karyotypes including trisomy 21, triploidy, Klinefelter syndrome, balanced and unbalanced autosomal translocations. The balanced rearrangements included 46, XX, t(4:6) (q31.3; q26.1) mat, 46, XY, t(2;11) (q23;p15) mat, 46, XX, t(8;13) (q24.3q14.1)mat, 46, XY, t(1;18) (p31; q21.3), 46, XY, t(4;9) (q25;q22) mat, and 46, XY, inv (7) (p22q11.23). Two of the cases had a bisatellited marker chromosome. The sex chromosome abnormalities were 47, XXY, 47, XXX, and 46, XY [[99]/47, XYY[15]/45, X[9]. The majority of abnormal cases (40%) were due to abnormal maternal serum screening test, followed by advanced maternal age (37%).
Conclusion: Our findings emphasis the importance of maternal serum screening for the detection of chromosome abnormalities in the fetus for at risk pregnancies.

Keywords: Amniocentesis, Karyotyping, Maternal Serum Screening, Trisomy 21, Parental Translocation

P-79: Effect of Salvia Hypoleuca on the cAMP-Responsive Element Modulator (CREM) Expression and Spermatogenesis in Rat

Estakhr J[1*], Hatami L[1], Shams Lahijani M[2], Sanchooli N[1], Nikravesh A[1]

1. Biology, Zabol University, Zabol, Iran
2. Anialm Science, Shahid-Beheshti University, Tehran, Iran
Email: j.estakhr@uoz.ac.ir

Objective: Salvia species which are medicinal herbs, have been used to improve male reproductive functions in traditional medicine. They also, have been used to treat several diseases. In this study, effects of Salvia hypoleuca on male rat reproductive function, by sperm analysis and assessments of CREM expression at mRNA and protein levels, were investigated.
Materials and Methods: S. hypoleuca (300 mg/kg/day) was administered to 10-week old male wistar rats for 56 consecutive days. Then sperm analysis and RT_PCR and western blotting were caried out.
Results: Results indicated significant increase in the weights of the testes, epididymal sperm counts, and sperm motility compared to control group. RT-PCR and western blotting analysis showed an increase in the expression of both CREM mRNA and protein levels.
Conclusion: These results suggest that S. hypoleuca induces spermatogenesis via CREM activation in rat testes and improve male fertility.

Keywords: CREM Expression, Spermatogenesis, Male Fertility, Saliva Hypoleuca

P-80: A study on the Existence of Inhibin - Subunit Gene Mutation in a Population of Iranian Women with Premature Ovarian Failure

Fallahian M[1*], Pouresmaeili F[2], Azizi F[3], Zali M.R[4], Samani E.N[1], Kharaziha P[4]

1. Gynecology Department, Shahid Beheshti University (MC), Tehran, Iran
2. Genetics Department, Shahid Beheshti University (MC), Tehran, Iran
3. Endocrinology Department, Shahid Beheshti University (MC), Tehran, Iran
4. Gastroenterology Department, Shahid Beheshti University (MC), Tehran, Iran
Email: fpoures@yahoo.com

Objective: Demonstration the candidate mutation as a gene variation associated with POF in Iranian population.
Materials and Methods: Using DNA sequencing, DNA samples were screened from 24 women with POF and 24 controls below 40 years old for mutations in the Inhibin gene.
Results: the 769G A mutation in exon 2 of the Inhibin-gene was found in four out of 24 idiopathic POF patients.
Conclusion: The results obtained in this study have shown that this variation is more frequent in patients

with POF than normal fertile populations of Iran.

Keywords: Inhibin - Subunit Gene, Premature Ovarian Failure, Mutation

P-81: Vascular Endothelial Growth Factor Gene Polymorphism and Ovarian Hyperstimulation Syndrome

Nasrin Ghasemi*, Razieh Firouzabadi, Shahnaz Ahmadi, Homa Oskouian

Yazd Research and Clinical Center for Infertility, Yazd, Iran
Email: ahmadishahnaz2005@yahoo.com

Objective: In this cross sectional study potential association between of VEGF gene 460, 1154, 405 polymorphisms and OHSS was evaluated
Materials and Methods: A total of 160 patients who were candidate for IVF/ICSI were enrolled in this study. Among them 80 patients had OHSS and 80 patients were normoresponse to ovulation induction All patients were stimulated by long agonist protocol. , one blood sample (5 ml) was collected from each patient. Genomic DNA was extracted from peripheral blood using phenol – cholorophem method. Polymerase chain reaction- restriction fragment length polymorphism analysis was used to analyse the VEGF 460, 405, 1154 genotype of OHSS patients and normoresponder controls.
Results: There was no significant differece in genotype frequency and allel frequency of 460 polymorphism between two groups. other result will be presented in congress.
Conclusion: The association of the VEGF 460 polymorphism and OHSS is weak. other result will present in congress.

Keywords: Vasular Endothelial Growth Factor, Vegf 460 Gene Polymorphism, Vegf 405 Polymorphism, Vegf 1154 Gene Polymorphism, OHSS

P-82: Study of Short Time Exposure to 4°C Temperature on Gene Expression Profile Monocarboxylic Transporter Genes MCT 1, 2, 3, 4 in 4-Cell Mice Embryo

Janan A[1]*, Ramezani M[2]

1. Developmental Biology, Payame Noor, Tehran, Iran
2. Embryology, Azad University, Ashtiyan, Iran
Email: arghvanjanan@yahoo.com

Objective: The aim of this study was to assay the expression profile of MCT1,2,3,4 in 4-cell Fresh and 4°C mouse embryoes.
Materials and Methods: NMRI female mice on a fixed light-dark cycle were exposured by interaperitoneal injection of 10 IU PMSG and followed 48 h later by 10IU hcG.injected mice mated with mature male NMRI mice. After view the vaginal plug,4-cell embryoesoe obtained from mice that killed by cervicaldislocation at 52-55 h post hcG injection respictievly.Embryoes separated to 2 groups:1. Fresh group consist of 4-cell embryos in KSOM.2. 4°C embryos in KSOM with 24 h incubation in 4°C.The presence of mRNA encoding MCT 1,2,3,4 at each group were demonstrated by RT-PCR kit on , and products of PCR loaded on 3% Agarose gel.
Results: MCT1 expressed in Fresh and 4◦C groups.neither MCT 2 nor MCT3,4 expressed in each 2 groups.
Conclusion: MCT1 have a major role in development of mouse embryo.It expression is same in Fresh and 4◦C mouse embryoes. Not only MCT2 but also MCT3,4 not expressed in Fresh and 4°C embryoes.

Keywords: 4°C Temperature, Transporter Genes MCT1, 2, 3, 4, Mice 4-Cell Embryo

P-83: Exploring the Role of Maternal KIR and Parental HLA-C Genotypes in the Maintenance of Pregnancy

Khosravifar M[3]*, Sanati M.H[2], Habibi R[1], Chaparzadeh N[3], Gourabi H[1]

1. Department of Genetics, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. National Institute for Genetic Engineering and Biotechnology, Tehran, Iran
3. Tarbiat Moalem of Azarbayejan University, Tabriz, Iran
Email: rhabibi_ir@yahoo.com

Objective: Recently, it has been proven that interaction between the uterine natural killer-cells and fetal trophoblasts is a key factor in successful implantation. In human, the trophoblast cells express less Polymorphic, non- classical MHC and classical HLA-C that is highly polymorphic. Interaction between HLA-C molecule and the receptors on uNK Cells result in release of a variety cytokines and chemokines that modulate placental relationship between mother and her fetus. Therefore investigation of parental HLA-C and maternal KIR genes is interesting for determining the role of these genes in occurrence of recurrent miscarriage.
Materials and Methods: We have screened the couples who have referred to Royan Institute for finding the cases of idiopathic abortion. We have done genotyping for maternal KIR/ parental HLA-C genes using the PCR-sequence-specific primer method and compare with control group who have normal pregnancy.
Results: HLA-C molecules recognized by KIR can be divided into C1 and C2 phenotype that discriminated by different amino acids at position 80. Accordingly, all of the control group were heterogeneous (C1\C2) for this locus and genotyping of patients is ongoing that will be presented after performing of analysis and compare to controls.
Conclusion: Whereas KIR /HLA-C genes intensively polymorphic, any of particular maternal KIR/parental HLA-C genotypes may have different effect on success of pregnancy. In order to access probable fetal HLA-C

genotype, determination of parental HLA-C genotypes is required for identifying the role of any HLA-C allotypes or its specific receptors on the uNK- cell function related to miscarriage.

Keywords: Recurrent Miscarriage, Uterine-Natural Killer Cells HLA-C Polymorphism, Killer - Immunoglobulin - Like Receptors

P-84: Y Chromosome Microdeletions in Idiopathic Infertility in Khuzestan

Konar E[1]*, Khatami S.R[1], Galehdari H[1], Mombeini H[2]

1. Genetic, Chahid Chamran University, Ahvaz, Iran
2. Urology, Golestan Hospital, Ahvaz, Iran
Email: ahmadkonar@gmail.com

Objective: Male infertility is believed to be associated with deletions on the Y chromosome as firstly has been reported by Tiepolo and Zuffardi. The microdeletions are commonly occurring in the specific region in the chromosome Y has been called as AZF, a region that thought to contain some genes are involving in spermatogenesis (Dada et al., 2003). The AZF region is subdivided into three non-overlapping sub-regions called AZFa in the proximal portion (interval D3-D6), AZFb in the intermediate region (D13-D16) and AZFc in the distal region (D20-D22) (Foresta et al., 2001). They are strongly associated with spermatogenic defects, such as azoospermia and oligozoospermia (Ferrás et al., 2004a; Foresta et al., 2005). Microdeletions in the AZF region are frequently found in patients with azoospermia. The incidence of these microdeletions has been found to vary from 3 to 55% in Yq of patients are diagnosed with infertility (Foresta et al., 1998; Vogt, 2004). Although a high percentage of infertile men with microdeletions in the Y chromosome are not able to produce children by natural mechanisms of reproduction, there can be transmission of the father's infertility problems to his sons, when they are produced by assisted reproduction. This predisposition for infertility can include gradual alterations in spermatozoid production, so that a young man with oligozoospermia later becomes azoospermic (Kihaile et al., 2005).
Materials and Methods: We examined microdeletions in the Y chromosomes of men with azoospermia and severe oligozoospermia in Khuzestan province. Thirty-one patients with azoospermia and 47 with severe oligozoospermia were analyzed by PCR. The patients were classified according to alterations detected in three consecutive spermograms, based on the WHO technique (1999), into groups with non-obstructive azoospermia and those with severe oligozoospermia (≤5 x 106 sperm/mL) and patients' blood were collected. Genomic DNA was extracted from peripheral blood lymphocytes and microdeletion analysis was made of the regions AZFb and AZFc sequence-tagged sites. The PCR product was run by electrophoresis on a 1.5% agarose gel impregnated with ethidium bromide at 5µg/mL and visualized under UV light.
Results: Among the 78 patients with azoospermia or severe oligozoospermia, 11 patients that have been diagnosed with severe oligozoospermia were positive for microdeletions, from them nine patients (21.2%) showed deletions in the AZFb region and two patients (4.2%) in the AZFc region.The ages of the azoospermic patients varied from 23 to 47 years, with a mean of 31 years. Patients with severe oligozoospermia ranged from 22 to 38 years, with a mean of 32 years.
Conclusion: We conclude that microdeletions in Yq could be one of the important causes of idiopathic male infertility and our findings support previous studies.

Keywords: Male Infertility, AZF, Azoospermia, Oligozoospermia, Y Microdeletions

P-85: Observation of Satellite Association in Couples with History of Habitual Abortion

Manoochehry F*, Razazian F, Mortezapour F, Rahnama M, Nasiri F, Mahjoubi F

Iranian Blood Transfusion Organization Research Center, Tehran, Iran
Email: s_sayar0483@yahoo.com

Objective: Here we report a very rare and interesting case with a constitutional break or gap at site (16q22.3). A couple refereed to our laboratory for chromosome study due to the history of miscarriage and still birth. The woman had a normal karyotype. The man had 4 cell lines. One cell line had a breakage at chromosome 16q22.3, one cell line with the deletion at 16 q22.3, one cell line with two normal chromosome 16 and a marker chromosome (very likely originated from 16q22.3). The last cell line had apparently normal male karyotype. To our best knowledge there are only two other case with the same break or gap at 16q22 resulting to abortions in both cases and a full term delivery with a Down syndrome child in one case. The phenotype of our case was normal because the cells with deletion and with marker of 16q22.3-qter outbalance each other. It is very likely that the deletion and marker cell lines emerged in culture and would die out in the next cycle. Thus, the major problem is that of spontaneous abortions. The couples advice to have genetic counseling and prenatal diagnosis for the future pregnancies.
Materials and Methods: Lymphocyte cultures from the patients were set up in RPMI1640 supplemented with 20% FBS.high resolution chromosome banding was performed in all subjec
Results: Thus, the major problem is that of spontaneous abortions. The couples advice to have genetic counseling and prenatal diagnosis for the future pregnancies.
Conclusion: The phenotype of our case was normal because the cells with deletion and with marker of 16q22.3-qter outbalance each other. It is very likely that the deletion and marker cell lines emerged in culture and would die out in the next cycle.

Keyword: Infertility

P-86: Association of Tumor Necrosis Factor-Alpha and Interleukin-10 Gene Polymorphisms in Iranian Patients with Pre-eclampsia

Mirahmadian M[1]*, Kalantar F[1], Heidari G[2], Afshin A[3], Safdarian L[4], Amirzargar A.A[1]

1. Immunology, Tehran University of Medical Sciences, Tehran, Iran
2. Radiology, University of California Los Angeles, Los Angeles, US
3. Johns Hopkins Bloomberg School of Public Health, Baltimore, US
4. Obstetrics, Tehran University of Medical Sciences, Tehran, Iran
Email: mirahmadian@sina.tums.ac.ir

Objectives: Considering that certain cytokines may change during pre-eclampsia (PE), because of functional polymorphisms in their genes, our purpose was to determine the association between tumor necrosis factor-alpha (TNF-alpha) and interleukin-10 (IL-10) gene polymorphisms and development of PE.
Materials and Methods: The genetic polymorphisms of TNF-alpha and IL-10 was studied by polymerase chain reaction sequence specific primers (PCR-SSP) in the DNA of PBC from 160 patients with PE and 100 healthy pregnant women. SPSS software, version 11.5, was used for statistical analysis. Association analysis was used in order to identify any relationship between the alleles and the occurrence of PE versus normal pregnancy in patients.
Results: We found a significant difference between TNF-alpha A allele (-308) and G allele (-238) in PE patients compared with those of the control groups. A significantly higher C⁄C genotype frequency of IL-10 (-592) and (-819) was observed in the PE patients than in the control groups. In addition, the frequencies of three common IL-10 haplotypes (GCC, ACC, and ATA) did not show any significant difference between the study groups.
Conclusion: These findings would support the concept of contribution of TNF-alpha and IL-10 gene polymorphisms in the pathogenesis of PE in our population.

Keywords: Genotype, Haplotype, Interleukin-10, Polymorphism, Pre-Eclampsia, Tumor Necrosis Factor-Alpha

P-87: A Novel Ring X in a Female with Secondary Amenorrhea

Mortezapour F*, Rahnama M, Nasiri F, Manoochehri F, Razazian F, Mahjoubi F

Iranian Bblood Transfusion Organization Research Center, Tehran, Iran
Email: f-elena1386@yahoo.com

Objective: A 18-year-old patient with secondary amenorrhea and normal sexual characteristics refereed to us for chromosome study. She was proved to have a karyotype 45, X[28] / 46, X, r (x) (p?;q?) [12]. She was premature delivered at 7 months. At the age she diagnosed to have this ring chromosome was mentally at normal range but physically and developmentally delayed. Our findings indicate the necessity for cytogenetic studies in certain cases of amenorrhea. This is a very rare karyotype in patients with secondary amenorrhea.
Materials and Methods: lymphocyte cultures from the patients were set up in RPMI1640 supplemented with 20% FBS.high resolution chromosome banding was performed in all subject.
Results: Our findings indicate the necessity for cytogenetic studies in certain cases of amenorrhea. This is a very rare karyotype in patients with secondary amenorrhea.
Conclusion: A 18-year-old patient with secondary amenorrhea and normal sexual characteristics refereed to us for chromosome study. She was proved to have a karyotype 45,X[28]/46,X,r (x) (p?;q?) [12]. She was premature delivered at 7 months. At the age she diagnosed to have this ring chromosome was mentally at normal range but physically and developmentally delayed.

Keyword: Ringx

P-88: Cytogenetic Analyses of Child with Paroxysm

Nasiri F*, Manouchehri F, Mortezapour F.R.F, Rahnama M, Zamanian M, Mahjoubi F

Iranian Blood Transfusion Organization Rcenter, Tehran, Iran
Email: nasiri_shirzad@yahoo.com

Objective: Investigation Here we report a male child with paroxysm referred to our laboratory for cytogenetic.
Materials and Methods: lymphocyte cultures from the patients were set up in RPMI1640 supplemented with 20% FBS.high resolution chromosome banding was performed in all subject.
Results: G banding revealed an apparently balanced reciprocal translocation between chromosome 4 and 10. The karyotype was ascertained as :46,XY,t(4;10) (q21;q26.2)
Conclusion: The patient was hyperactive but mentally normal. The parents had first degree consanguineous marriage. Chromosom analysis was performed on preparation made from peripheral blood using standard protocol. G banding revealed an apparently balanced reciprocal translocation between chromosome 4 and 10. The karyotype was ascertained as :46,XY,t(4;10) (q21;q26.2) The parents had normal karyotyepe. The abnormality present in this child could be caused by the possible deletion of some of the important genes

located on regions involved in these breakpoints or it can be just a coincidence

Keywords: Paroxysm, Translocation, Familial Marriage

P-89: DNA Methylation Patterns of bcl2, bax, bag1 and casp3 Genes in Fragmented and Normal Human Embryos Derived from ART

Piltan A[1]*, Eftekhari-Yazdi P[1], Toutounchi M[2], Gourabi H[2], Valojerdi M.R[1], Karimian L[1], Esalaminejad M.B[3]

1. Department of Embryology, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Genetics, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: peyzadi@yahoo.com

Objective: Fragmentation is common event observed in more than 50% human embryos that grows in vitro culture. The main mechanism of fragmentation is apoptosis. To achieve the correlation between apoptosis and fragmentation, we study the role of DNA methylation in anti-pro apoptotic genes expression inhibitation and its final effect on fragmentation.
Materials and Methods: Fragmented and normal human 8-cell embryos were scored according to the degree of fragmentation , into four grades (grade I: normal or least fragmentation embryos, grade II: embryos with lower than 25% fragmentation, grade III: embryos with more than 25% fragmentation, grade IV: embryos to induced with a apoptotic inducer Actinomycin D). In this study, TUNEL labeling was used to detect apoptosis, also Bisulfite-Sequencing Technology characterized methylation status of the bag1, bcl2, casp3 and bax enhancer/promoter regions, and then Real-Time PCR. confirmed analysis of gene expression in human embryos.
Results: The results of TUNEL labeling showed that embryos with higher fragmentation had a high number of apoptotic bodies. Bisulfite sequencing and quantitative PCR analysis were used respectively to indicate the level of gene expression and DNA methylation profiles of the above genes in four different embryo grades. To determine DNA methylation changes between these embryo grades, we analyzed the CpG islands states of various regulatory regions of bag1, bax, bcl2, casp3 and bax genes.
Conclusion: The primery data revealed that bax enhancer/promoter region was hypomethylated through grade I to IV whereas it seems that methylation of Bag1 varied between these embryo grades, the finding should be confirm by their expression level that is ongoing.

Keywords: Embryo Fragmentation, Apoptotic Genes, DNA Methylation, Bisulfate Sequencing

P-90: A Report of A Case with Partial Trisomy 13q with 46, XY, Der(14) t(13; 14) (q13; p11.1), +13q13 Karyotype

Rahnama M, Nasiri F, Manoochehry F, Razazian F, Mortezapour F, Mahjoubi F

Iranian Blood Transfusion Organization Research Center, Tehran, Iran
Email: miti_raft@yahoo.com

Objective: This has been shown that the trisomy of the distal part of chromosome 13 is related to different clinic findings than cases with classic trisomy 13. The different trisomic segments of the long arm of chromosome 13 have been reported which might be either translocated or inserted in different chromosomes. Our case was a baby boy and the first child of an unrelated family. He was born at term following a normal pregnancy. He referred to our clinic at the age of 4 months. He had postaxial polydactyly and syndactyly of the left hand. He was deaf and legally blind. He was generally presented with developmental delay, microcephaly, trinogocephaly, hypotelorism, and with feeding problem. Cytogenetic analysis carried out using Tripsin Giemsa G banding (GTG), the karyotype 46,XY,der(14)t(13;14)(q13p11.1)+13q13 was determined in our patient . His mother and father were investigated and found to have normal karyotypes. To our best knowledge, this is the first report of a case where the trisomic segment of chromosome 13 is translocated on to chromosome 14.
Materials and Methods: lymphocyte cultures from the patients were set up in RPMI1640 supplemented with 20% FBS.high resolution chromosome banding was performed in all subject.
Results: To our best knowledge, this is the first report of a case where the trisomic segment of chromosome 13 is translocated on to chromosome 14.
Conclusion: Our case was a baby boy and the first child of an unrelated family. He was born at term following a normal pregnancy. He referred to our clinic at the age of 4 months. He had postaxial polydactyly and syndactyly of the left hand. He was deaf and legally blind.

Keyword: Trisomy 13q

P-91: Cytogenetic Effects of Gonadotropin Releasing Hormone Analogue: Buserelin During Ovulation Induction Cycle by Sister-Chromatid Exchange Assay

Rahimian M[1]*, Gourabi H[2], Madani T[3], Behjati F[4]

1. Biology Departmetn, Research Branch, Azad University, Tehran, Iran
2. Department of Genetics, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Endocrinology and Female Infertility, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
4. Genetics Department, Social Welfare and Rehabilitation

University, Tehran, Iran
Email: rmouness@yahoo.com

Objective: Increasing use of Assisted Reproductive Technologies (ART) ,make the investigation on its complications more necessary. One of the main concerns in this regard is the side effects of medications required for controlled ovarian stimulation. Systemic and local reactions to the medications, Ovarian Hyper Stimulation Syndrome (OHSS) and genetic changes which can lead to cancer must be checked in treatment of infertile patients. In this study, cytogenetic effect of Buserelin, which is a gonadotropin hormone agonist (GnRH agonist), has been evaluated during In-Vitro Fertilization (IVF) cycle.
Materials and Methods: Blood samples were taken from 40 females refer to Royan institute(25 females in ART cycle use Buserelin from day 15 to day 2 of second menstrual cycle and 15 normal fertile women as control), cultured and examined by Sister Chromatid Exchange (SCE) assay, considered as the most sensitive mammalian system for measuring the effects of mutagenic carcinogens. Also the changes of 17β-stradiol (E2), which is known to produce adverse effects such as embroyotoxicity, teratogenicity and carcinogenicity by DNA damaging, were evaluated in days 15 and 2 of menstrual cycles of both patients and control group.
Results: In both groups, evaluation of SCE frequencies demonstrates lower rate around early follicular phase as compared to ovulation time, because of lower dosage of E2. More decreased SCE frequency was observed after Buserelin injection because of its inhibited effect on E2, which result to grater decrease in E2 dosage.
Conclusion: Finally, results indicated that Buserelin doesn't seem to have a significant potential for induction of malignancies after ovulation induction treatment and other medications in ovulation stimulation must be checked.

Keywords: Assisted Seproductive Technologies (ART), Ovarian Hyper Stimulation Syndrom (OHSS), Buserelin, Sister Chromatid Exchange (SCE) assay, 17β-Stradiol (E2)

P-92: Zeta Sperm Selection: A Suitable Method for Recovery of Sperm with Low DNA Fragmentation and Protamine Deficiency

Razavi S[1]*, Khajavi N[1], Nasr Esfahani M.H[2], Tavalaee M[2]

1. Department of Reproduction and Development, Royan Institute for Animal Biotechnology, ACECR, Isfahan, Iran
2. Embryology and Andrology, Royan Institute, Isfahan, Iran
Email: razavi@med.mui.ac.ir

Objective: Currently selection of human sperm for ICSI is based on, motility and morphology but the sperm shape is an inadequate parameter and other procedures should be used for selection of normal sperm. Therefore the aim of this study was to compare the efficiency of Zeta method with Density gradient centrifugation (PurSperm) for separation of sperm with normal chromatin structure
Materials and Methods: Semen samples were obtained from 63 patients referred to Isfahan Fertility and Infertility Center. Sperm recovered from zeta method and Density gradient centrifugation (DGC) procedures were evaluated with respect to control (neat semen) group for protamine deficiency, by using Chromomycin A3 (CMA3) staining and for DNA integrity using three different techniques including: sperm chromatin dispersion test (SCD), Acridine orange (AO) ,and TUNEL assay.
Results: The results show that the percentage of CMA3 positive sperm have significantly reduced in Zeta and DGC procedures compared to the neat semen . In addition, using three different techniques ,the percentage of DNA damaged sperm have significantly reduced in both procedures compared to the neat semen (control) ($p < 0.001$)but, the percentage of DNA damaged sperm is significantly lower in the Zeta procedure compared to the DGC procedure with respect to the three tests. The results indicate that the efficiency of Zeta method to separate sperm with normal protamine and intact DNA was higher than DGC procedure
Conclusion: It can be concluded that Zeta method is more efficient to recover sperm with minimal DNA damage; however, this method has its own limitations.

Keywords: Sperm Selection, Zeta Method, Pure Sperm, DNA Fragmentation

P-93: Sperm Chromatin Structure and DNA Damage Induced by Malathion is Related to Oxidative/ Nitrosative Stress

Amin Rezvanfar M*, Shojaei Sadee H, Rezvanfar MA, Abdollahi M

Laboratory of Toxicology, Faculty of Pharmacy, and Pharmaceutical Sciences Research Centre, Tehran University of Medical Sciences, Tehran, Iran
Email: amin2_dvm80@yahoo.com

Objective: Malathion is widely used as a potent pesticide in many countries and has been shown to produce some adverse health effects via induction of oxidative stress in through the generation of free radicals and alteration of the cellular antioxidant defense system. The aim of this study was to investigate malathion-induced oxidative DNA injury in rat sperm by measuring the activities of plasma peroxynitrite (ONOO–), glutathione peroxidase (GPx), superoxide dismutase (SOD), Mn-SOD, catalase (CAT), and lipid peroxidation (LPO) in rats.
Materials and Methods: 12 male rats of 220-230 g were divided into two groups as follows: group 1 received only distilled water, group 2 received malathion (200 mg/kg/day) dissolved in distilled water. The

groups were treated for 7 days by intraperitoneal injection. To evaluate sperm chromatin quality and DNA integrity, Aniline blue and Acridine orange staining were done in the groups.
Results: In the blood plasma, the LPO, ONOO– and GPx were higher in the malathion group as compared with controls. Also plasma CAT decreased in malathion-treated animals comparing with controls. Malathion did not alter plasma total SOD and Mn-SOD. 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: The present results highly support the idea that sperm DNA damage of malathion is mediated through oxidative stress.

Keywords: Malathion, Lipid Peroxidation, Oxidative Stress, Sperm, DNA Damage

P-94: Role of Mitochondria in Repeated Pregnancy Loss

Seyedhassani S.M[1, 2]*, Hushmand M[1], Kalantar S.M[2], Hashemi gorji F[1], Aflatunian A[2]

1. National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
2. Research and Clinical Center for Infertility, Yazd, Iran
Email: sayedhassani@yahoo.com

Objectives: Pregnancy loss is the most common complication of pregnancy. About 1 in 300 couples involve with Repeated Pregnancy Loss (RPL) and the main part of them remains unknown. Apoptosis plays a role in early human development and embryonic loss.The aberrant expression of apoptotic related genes is seen in RPL. It seems internal apoptotic pathway and mitochondria as a main core of it, have important role in fertilization and proliferation of the cells. Mitochondrial DNA (mtDNA) is not transmitted through nuclear DNA (nDNA), and in most multicellular organisms, virtually all mitochondria are inherited from the mother's ovum, as it is unusual for sperm cells to contribute mitochondria when fertilizing ova. Bax is an important nuclear gene in mitochondrial pathway of apoptosis.The protein encoded by this gene belongs to the BCL2 protein family. This protein forms a heterodimer with BCL2, and functions as an apoptotic activator. We believe that the mitochondria, Bax and Bcl2 genes are good candidate for investigation of pregnancy loss .
Materials and Methods: 335 consecutive cases were studied. Genetic counseling, clinical, paraclinical, and cytogenetic studies were done for each couple. We analyzed the familial pedigree of them and then screened the idiopathic cases. In total 96 females who were suffered from idiopathic RPL. 1- Four multiplex PCR are done on each sample for detection of mitochondrial deletions. 2- Mitochondrial D-loop part consisting of the hyper variable regions is analyzed by PCR-sequencing method. 3- Bax gene is evaluated by PCR-sequencing method for promoter region and all seven exons. 4- Bcl2 gene is evaluated by PCR-sequencing method for promoter region and PCR-SSCP for the exons.
Results: 1- No mitochondrial deletions were found in 96 DNA samples. 2- Mononucleotide repeat (poly C) from 303 to 315 nucleotide positions (D310) exhibited a polymorphic length variation and mutations (C ins. in 37,CC ins. in 8 ,CCC ins. in 1,T-CCC in 1 , T-CCCC in 1, and T-CCCCC in 1 female) 3- D-loop region was evaluated by direct sequencing and we found 166 different variations in our study population. Among them, 95 variations were seen in RPL cases , 28 in control samples , and also 43 in both of them . 4- Change of A to G in promoter region of Bax gene was seen at nt. -55 in 93 females (96.87%) .
Conclusion: 1- Because of oxidative stress is one of the important cause of mtDNA deletions we suggest that this phenomenon seems is less involving in pregnancy loss. 2- Some of these nucleotide alterations might be involved in repeated pregnancy loss and could be included in a panel of molecular biomarkers for susceptibility in pregnancy loss and even failure of in-vitro fertilization. 3- A high rate of mutation in mitochondrial DNA in the D loop was found in samples from patients with RPL relative to healthy controls . 4- In seven SNPs that were found in case and control groups, we found significant difference between groups ($P<0.05$) (T16126C, T16189C, C16223T, C16294T, T16311C, T16362C, T16519C) . From the RPL group mutations, 15 SNPs were significant and four mutations was novel (A503G, A335G , T217C , C114ins.) 5- We believe that mutation in Bax gene will lead to early apoptosis. 6- The results can be used in assessment of RPL and probability of interventional treatment for improving of fertilization in ART methods.

Keywords: Mitochondria, Repeated Pregnancy Loss, Bax, Bcl2

P-95: Clinical Feutures of a Case with Eing Chromosome 18

Zamanian M*, Nasiri F, Mortezapour F, Manouchehri F, Razazian F, Rahnama M, Mahjoubi F

Iranian Blood Transfusion Organization Research Center, Tehran, Iran
Email: mahsa_emad@yahoo.com

Objective: Chromosome 18 Ring is a rare disorder in which there is loss (deletion) of genetic material from one or both ends of the 18th chromosome and joining of the chromosomal ends to form a ring. Associated symptoms and findings may vary greatly in range and severity from case to case, depending upon the amount and location of lost genetic material and other factors. A ring may also be formed without the loss of any genetic material

Materials and Methods: Lymphocyte cultures from the patients were set up in RPMI1640 supplemented with 20% FBS.high resolution chromosome banding was performed in all subject.
Results: Karyotyping after lymphocyte culture at the age of 14 months revealed 46,XX,r(18)(q21.2qter). The parent had normal karyotype. The clinical feature of our case is mostly compatible with the other reported cases of r(18) except the presence of abnormal teeth and heart problem. This report further contribute to to the clinical of the r(18).
Conclusion: Here we report an additional case of a 14 months girl with r (18). The girl was born at term after an uncomplicated pregnanacy and delivery. Birth weight was about 1.5 kg, length 48cm, and head circumference 36cm. The girls presented hypertelorism, hypotonia, epicanthal folds, abnormal fingers, low set ears, and abnormally growth teeth. Echocardiography indicated dilation of the aorta. Karyotyping after lymphocyte culture at the age of 14 months revealed 46,XX,r(18)(q21.2qter). The parent had normal karyotype. The clinical feature of our case is mostly compatible with the other reported cases of r(18) except the presence of abnormal teeth and heart problem. This report further contribute to to the clinical of the r(18).

Keywords: Ring, Chromosome

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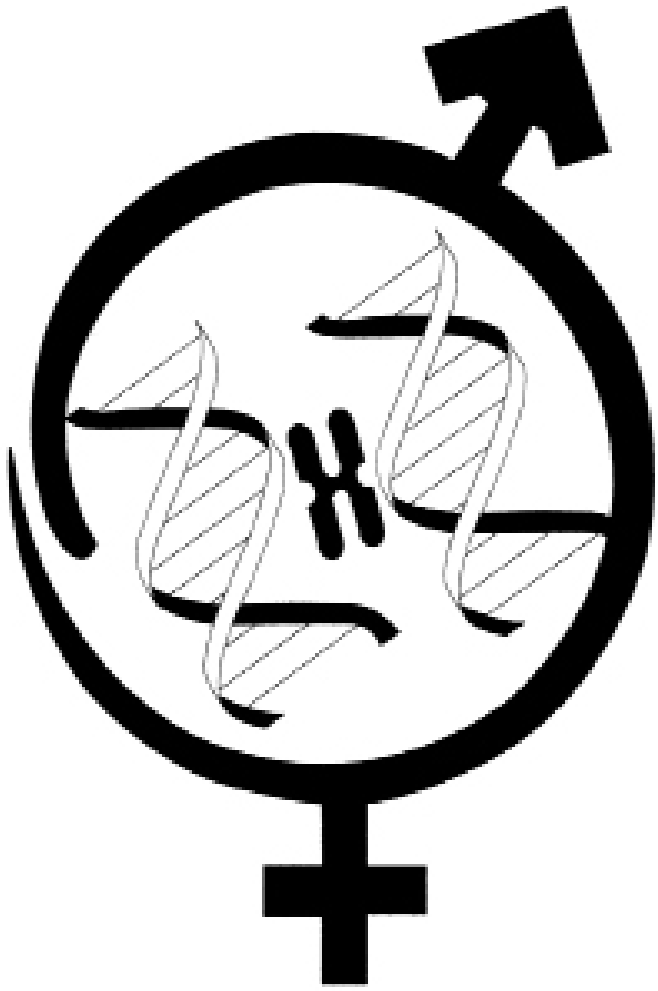

ROYAN INSTITUTE

Stem Cells

Is-1: The Culture and Transplantation of Human Limbal Stem Cells

Sajjad Ahmad

Centre for Stem Cell Biology and Developmental Genetics, University of Newcastle upon Tyne, Central Parkway, Newcastle upon Tyne, UK
Email: Sajjad.ahmad@ncl.ac.uk

The cornea is the clear front of the eye and is important for the transmission of light to the retina at the back of the eye for visual perception. The surface of the cornea is composed of an epithelium and this is maintained by stem cells located at the edge of the cornea, in a region known as the limbus. These so-called limbal stem cells can be lost, most commonly from chemical burns to the eye, resulting in the painful and blinding disease of limbal stem cell deficiency. In this disease, the normal corneal epithelium cannot be maintained and the corneal surface becomes covered by the surrounding phenotypically different conjunctival epithelium and its blood vessels. Recent advances have enabled us to treat severe limbal stem cell deficiency using culture expanded human limbal epithelium. Original culture techniques for human limbal epithelium require the use of animal cells or products within the culture system. We have developed an animal cell and product free culture system for human limbal epithelium. We are currently performing clinical trials on patients with unilateral limbal stem cell deficiency by transplanting autologous animal free cultured limbal epithelium. Using objective and subjective outcome parameters, the results from this ongoing trial seem promising to date.

Is-2: The Differentiation of Human Embryonic Stem Cells towards the Corneal Epithelial Lineage

Kenneth P McNatty

Sajjad Ahmad

Centre for Stem Cell Biology and Developmental Genetics, University of Newcastle upon Tyne, Central Parkway, Newcastle upon Tyne, UK
Email: Sajjad.ahmad@ncl.ac.uk

In order to identify an alternative source of human limbal stem cells, we have investigated the differentiation potential of human embryonic stem cells towards limbal stem cells and corneal epithelial cell lineage. By replicating the niche for adult human limbal stem cells, we have successfully differentiated human embryonic stem cells towards the corneal epithelial lineage. Collagen IV in the basement membrane and medium conditioned by limbal fibroblasts was used in the differentiation protocol. Our results show that, although within a three week period human embryonic stem cells can be differentiated towards limbal stem cells and the corneal epithelial lineage, the differentiation process seems incomplete. We have therefore termed the resulting cells "corneal epithelial like cells". As a by-product of this culture system, cells expressing differentiated skin epithelial cell markers were also identified. Because two different human embryonic stem cell lines were used in these studies, we have also discovered that the two different cell lines have a different capacity to differentiate towards the corneal epithelial lineage.

Is-3: Personalized Medicine: From Myth to Reality

Seiamak Bahram

Human Molecular Immunogenetics, Centre de recherché d'Immunologie et d'Hematologie, 4 rue Kirschleger, 67085 Strasbourg Cedex, France
Email: siamak@hemato-ulp.u-strasbg.fr

Throughout the past fifty years the study of inherited diseases has come of age. From purely cataloguing rare, Mendelian phenotypes, genetics and in fine medicine is now ready to tackle the genetics of complex diseases afflicting this time a considerable fraction of the population. These include cardiovascular, metabolic, neuropsychiatric as well as several infectious diseases. This intrusion of genetics in "everyday life" will have beside obvious scientific benefits, profound health, healthcare, societal, biotech and business implications which collectively will define a major medical revolution epitomized by the advent of personalized medicine (PM). In a few words PM could be defined as a process whereby each patient shall be diagnosed, monitored and eventually treated not only based on disease identification but the individual's proper manifestations chiefly dependant on the patient's genetic constituency. PM has therefore major implications for therapeutic intervention, mainly drug therapy - where the active drug will be tailored to patient's individual needs hence avoiding (sometimes dramatic) side effects not to mention needless medical expenses. This is not a fictional picture of the future as the fundamentals of this new medical revolution are already at hand and being implemented in various parts of the world and it is an understatement to say that within a period of less than one decade few diseases and therapies will "escape" the trend e.g. most drugs will be marketed based on the patient's genetic profile. During the presentation a few achievements of PM with regards to immune diseases will be highlighted.

Is-4: Genetic Dissection of Pluripotency in Human Embryonic Stem Cells

Ali Brivanlou

Rockefeller University, 1230 York Avenue, New York, NY 10065, USA
Email: brvnlou@rockefeller.edu

Is-5: Combining Reprogramming with Gene Therapy toward the Development of Cell-Based Therapy

Ali Brivanlou

Rockefeller University, 1230 York Avenue, New York, NY 10065, USA
Email: brvnlou@rockefeller.edu

Is-6: Vitamins E and D3 attenuate Demyelination and potentiate Remyelination Processes of Hippocampal Formation of Rats following Local Injection of Ethidium Bromide; Possible Role for Endogenous Stem Cells

Goudarzvand M[1], Javan M[1]*, Mirnajafi-Zadeh J[1], Mozafari S[1], Tiraihi T[2]

1. Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
2. Department of Anatomy, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
Email: m_javan@modares.ac.ir

CNS: Central Nervous System; EB: Ethidium Bromide; MS: Multiple Sclerosis; MBP: Myelin Basic Protein; OPCs: Oligodendrocyte Precursor Cells; SVZ: Sub-Ventricular Zone; EAE: Experimental Autoimmune Encephalomyelitis; DG: Dentate Gyrus

Is-7: Bone Marrow Cell Therapy for Osteogenesis Imperfecta

Edwin M Horwitz

Director of Cell Therapy, Associate Professor of Pediatrics, The Children's Hospital of Philadelphia, Abramson Research Center, Philadelphia, PA 19104, USA
Email: horwitze@email.chop.edu

Is-8: The Osteogenic Potential of Bone Marrow Stem Cells

Edwin M Horwitz

Director of Cell Therapy, Associate Professor of Pediatrics, The Children's Hospital of Philadelphia, Abramson Research Center, Philadelphia, PA 19104, USA
Email: horwitze@email.chop.edu

Is-9: Absence of Tumor Outcome after Autologus Bone Marrow (BM) Stem Cell Transplantation for Liver Cirrhosis

Malekzadeh R[1]*, Bagheri M[1], Alimoghaddam K[2], Mohamadnejad M[1], Baharvand H[3, 4, 5], Ghavamzadeh A[2]

1. Digestive Disease Research Center, Tehran University of Medical Sceinces, Tehran, Iran
2. Bone Marrow Transplantation Research Center, Tehran, Iran
3. Department of Stem Cells and Developmental Biology, Royan Institute Stem Cells Biology and Technology, ACECR, Tehran, Iran
4. Department of Regenerative Medicine, Royan Institute Stem Cells Biology and Technology, ACECR, Tehran, Iran
5. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran
Email: malek@ams.ac.ir

Objective: Stem cell therapy may offer new hope in the management of cirrhosis. One of the potential complications of stem cell therapy is tumor development. Recently, a case of donor-derived brain tumor has been reported after transplantation of neural stem cells (Amariglio N, et al. PLoS Medicine 2009). There is no long term follow up study in regard to the tumor development in cirrhotic patients who underwent stem cell transplantation. we aim to report the data related to the tumor development in our three prospective trials of autologous stem cell transplantation in liver cirrhosis.
Materials and Methods: Data of serum alfa-fetoprotein (AFP) levels, abdominal ultrasound, and abdominal CT scan at baseline and at the end of follow up were assessed from the databank of the following prospective trials.A phase 1 trial(1) in which the mean number of 7 milion CD34+ hematopoietic BM stem cells were infused through hepatic artery in 3 patients with cirrhosis. Another phase 1 trial(2)in which the mean number of 31 milion mesenchymal stem cells (MSC) were infused through peripheral vein in 4 patients. A phase 2 trial(3) in which the mean number of 400 milion MSC were infused through peripheral vein in 8 patients. The last study is an ongoing randomized controlled trial, which the data of the treatment arm is presented. Serum AFP levels, and abdominal ultrasonography were done every 6 months for all the patients. Also, all of them had baseline CT scan, and follow up CT scan at 6 months of post-transplantation. All the patients were on the waiting list of liver transplantation.
Results: A total of 15 patients (6 men) were evaluated. Mean age of the patients was 45.1 (± 14.9). Mean duration of follow up was 23.5 (±9.7) months. Mean serum AFP was 2.8 (± 1.9) mcg/L at baseline, and 4 (± 1.5) mcg/L at the end of follow up ($p > 0.05$). No evidence of liver tumor, or other intra-abdominal tumors was found in the 15 studied patients during the study period. Four out of 15 patients (e.g. 26.7%) died due to complications of cirrhosis during the follow up. None of the patients underwent liver transplantation.
Conclusion: In this long term follow up study, we found no evidence of tumor development in cirrhotic patients who underwent autologous BM stem cell transplantation.

Is-10: Cardiomyocytes from Human Pluriptoent Stem Cells in Development and Drug Discovery

Christine Mummery

Department of Anatomy and Embryology, Leiden University Medical Centre, The Netherlands
Email: C.L.Mummery@lumc.nl

There is an urgent unmet need for reliable cardiac safety pharmacology assays to identify potential risks early in drug development and reduce time and cost to market. Human pluripotent (embryonic) stem cells (hESC) are a renewable, scalable and reproducible source of cardiomyocytes (CM) on which to base such test systems. Microarray analysis of modulations in gene expression during differentiation has shown that the major known cardiac genes are upregulated but that novel genes are also expressed. Normal cardiac development is recapitulated in vitro.
Here we describe the field potential of hESC-CM, measured using commercially available multi electrode arrays. Systematic generation of dose response curves for cardiac and non-cardiac drugs show that hESC-CM accurately predicts reported drug effects on the human heart. These include blocking the human Ether-a-go-go Related Gene (hERG) ion channel, resulting in QT prolongation; this is associated with life-threatening arrhythmias, such as Torsade de Pointes (TdP). On this basis, we propose two directly applicable safety criteria for pre-clinical evaluation of new drugs in development: (1) prolongation of field potential duration (FPD) and (2) sodium peak reduction. This is the first study in which dose responses of such a wide range of compounds have been compared in hESC-CM and the outcome shown to predict clinical effects. We propose that assays based on hESC-CM could complement or potentially replace some of the preclinical tests currently used to select chemical compounds for development as new cardiac drugs and improve safety confidence once in clinical use.

Is-11: Human Embryonic Stem Cell Derived Cardiomyocytes in Heart Repair

Christine Mummery

Department of Anatomy and Embryology, Leiden University Medical Centre, The Netherlands
Email: C.L.Mummery@lumc.nl

Derivation of heart cells from human embryonic stem cells (HESCs) and understanding the underlying developmental mechanisms is the main focus of the research. Culture conditions have now been sufficiently refined that cardiomyocyte differentiation is an efficient and reproducible process. Microarray analysis of modulations in gene expression during differentiation has shown that the major known cardiac genes are upregulated but that novel genes are also expressed.
Genetically marked HESCs have been produced in which expression of the green fluorescent protein marker is retained after differentiation. This has permitted unambiguous tracing of cardiomyocytes following transplantation into a mouse heart. Long term survival of the cells and integration into the host heart has been observed and the ability of these cells to restore cardiac function in mice that have undergone myocardial infarction is being investigated.

Is-12: Quantitative Phospho-Proteomics in Early Stem Cell Differentiation

Javier Muñoz[1, 7], Dennis Van Hoof[1, 2, 3, 7], Stefan R. Braam[2, 4, 7], Martijn W.H. Pinkse[1, 5, 7], Rune Linding[6], Albert J.R. Heck[1], Christine L. Mummery[2, 4], Jeroen Krijgsveld[1, 8]

1. Biomolecular Mass Spectrometry and Proteomics Group, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Sorbonnelaan 16, 3584 CA Utrecht
2. NetherlandsDevelopmental Biology and Stem Cell Research, Hubrecht Institute, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands
3. Diabetes Center, University of California San Francisco, 513 Parnassus Avenue, San Francisco, CA 94143, USA
4. Department of Anatomy and Embryology, Leiden University Medical Center, Einthovenweg 20, 2333 ZC, The Netherlands
5. Analytical Biotechnology Group, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands
6. Cellular & Molecular Logic Team, Institute of Cancer Research (ICR), Section of Cell & Molecular Biology, 237, Fulham Road, London, United Kingdom
7. These authors contributed equally to this work
8. EMBL, Gene Expression Unit, Meyerhofstrasse 1, 69117, Heidelberg, Germany
Email: jeroen.krijgsveld@embl.de

Pluripotent embryonic stem cells (ESC) can self-renew indefinitely and possess characteristic protein-protein networks which remodel during differentiation. How this is controlled at the protein and protein-signalling level is poorly understood. To study the networks and cascades activated by phosporylation in the early phase of differentiation, we adopted a quantitative proteomic approach in ESC, combining SILAC labeling and selective capturing of phospho-peptides.
Embryonic stem cells were SILAC-labeled with Lys and Arg. Unstimulated labeled cells were mixed with unlabeled cells that had been incubated with bone morphogenetic protein (BMP) for 30, 60 or 240 minutes to induce differentiation. From each of these combined samples, proteins were digested and phosphopeptides were enriched by subsequent SCX and on-line TiO2 chromatography. Proteins were identified using Mascot, and quantified using MSQuant.
In total, 144 LC-MS runs were performed using LTQ-

FT and LTQ-orbitrap mass spectrometry, collecting over 800,000 peptide spectra. Over 2000 phosphopeptides were identified in each of the 3 datasets, totaling over 3600 unique phosphopeptides across all samples. Next, phosphorylation levels were quantified across the 3 time points, and data were analysed at various levels. First, we have followed the dynamics of phosphorylation over time at the peptide level, since for most peptides we have mapped the phosphorylation site. Indeed we have found many different profiles, which often varied foro individual phosphorylation sites in the same protein. Second, we have deduced kinase motifs enriched in our dataset, based on conserved regions around phosphorylation sites using NetworKIN. This identified CDK1/2 as a central kinase in the activated network. Third, we have mapped (differential) phosphorylation to signaling pathways by using gene ontologies and prior knowledge of protein-protein and biochemical interactions. This has indicated the activation of several signaling cascades, including Jnk and Akt. Finally, we have identified 26 proteins that are widely used as stem cell markers because they are associated with the undifferentiated state of ESC. In 12 of these proteins, 30 phosphorylation sites were identified, of which 25 were unknown so far. This could indicate that (de)phosphorylation of these proteins could be associated with the induction of differentiation. Currently we are integrating these combined data in a model that describes the events in the initial phase of ESC differentiation.

Is-13: Clinical Liver Cell Therapy-State of the Art

Michael Ott

Hannover Medical School, Department of Gastroenterology, Hepatology and Endocrinology and Twincore Centre for Experimental and Clinical Infection Research, Foedor Lynen – Str. 7-9, 30625 Hannover, Germany
Email: Ott-mhh@gmx.de

The liver has adapted to the inflow of ingested toxins by the evolutionary development of unique regenerative properties and responds to injury or tissue loss by rapid division of the mature cells. Proliferation of the parenchymal cells, i.e. hepatocytes and bile duct epithelial cells is regulated by numerous cytokine/growth factor mediated pathways and synchronised with extracellular matrix degradation and restoration of the vasculature. Hepatocytes, which have been infused into the liver vasculature, do engraft in small numbers and can participate in the regeneration process. In animal models with a selection advantage transplanted hepatocytes can grow to large numbers and may substitute the recipient liver mass completely.
Since the first hepatocyte transplantations in animals, a number of reports have appeared showing the beneficial effects of hepatocyte transplantation in different animal models and also in human liver disease. In the clinic hepatocyte transplantation is being tested for the treatment of metabolic liver disease and to decrease mortality in acute liver failure. We have applied hepatocyte transplantation in four children with severe neonatal urea cycle defects (UCD). UCD is considered a promising target disease for liver cell transplantation (LCT), which may be a less invasive alternative or supplementation to orthotopic liver transplantation. Cryopreserved hepatocytes were isolated under good manufacturing practice conditions. The patients (age 1 day - 3 years) received multiple intraportal infusions of cryopreserved hepatocytes from that same donor, a 9-day old neonate. Portal vein access was achieved surgically in two children, whereas the umbilical vein was suitable for interventional catheter placement in two neonates. All children showed metabolic stabilization during observation periods of 4 to 13 months. One child with prenatally diagnosed ornithine transcarbamylase deficiency died after 4 months from a fatal metabolic decompensation.
Although hepatocytes can be obtained from liver resection in live donors, or from cadaveric liver donors, one of the major impediments to more widespread use of hepatocytes to treat liver disease is a significant shortage of hepatocytes suitable for cell transplantation. For this reason, different sources of hepatocytes other than those primarily isolated from adult livers are being investigated not only to be used for clinical transplantation but also to investigate the potential of liver cell therapy. Since the capacity to produce liver cells for experimental and therapeutic use is limited, stem cells of various origins have been studied as a renewable source of liver cells. In vitro induction of hepatic phenotypes has been demonstrated in embryonic and adult stem cells and "proof of principal" transplantations have been performed. However, little is known yet about the relative repopulation and tissue forming capacity of these cells in a controlled setting compared to primary adult hepatocytes. We demonstrate that hepatocytes with an adult phenotype show the highest capacity to repopulate a mouse liver. We will discuss the implications for the development of new differentiation protocols and the requirements for stem cell based therapies in liver diseases.

Is-14: ES/iPS Technology and Generation of Liver Cells

Michael Ott

Hannover Medical School, Department of Gastroenterology, Hepatology and Endocrinology and Twincore Centre for Experimental and Clinical Infection Research, Foedor Lynen – Str. 7-9, 30625 Hannover, Germany
Email: Ott-mhh@gmx.de

Several stem cell sources have been studied extensively for their potential to differentiate into cells of the hepatic phenotype in the last years. However, most of the currently available data indicate that somatic cell types

have a limited capacity to generate liver tissue in transplantation experiments. Embryonic stem (ES) cells can be maintained in a state of pluripotency for long periods of time, and can be grown in large numbers. Spontaneous differentiation of ES cells can be achieved through the formation of embryoid bodies (EB) and subsequent inoculation of the EB-derived cells on adherent matrices facilitates differentiation into hepatocyte-like cells in the presence of a variety of growth factors, cytokines and hormones, such as hepatocyte growth factor (HGF), fibroblast growth factor (FGF) and dexamethasone. Significant progress has been made in identifying factors and developing protocols to enforce the differentiation of ES cells into cells of the hepatocyte lineage.
Hepatocyte formation derived from sorted ES-HPC has recently been demonstrated in fumaryl-acetoacetate-deficient (FAH-/-) and uroplasminogen-activator transgenic MUP-uPA/SCID mice, clearly indicating the potential for cellular therapies. The rate of hepatocyte and liver tissue formation, however, was much lower than that previously reported for transplanted primary adult hepatocytes or fetal hepatoblasts. The reduced efficacy of hepatocyte and liver tissue formation may have been due to lower primary engraftment rates of the ES-HPC into the recipient liver, incomplete differentiation, or a combination of both.
Induced pluripotent stem (iPS) cells are the product of somatic cell reprogramming to an embryonic-like state. This occurs by the introduction of a defined and limited set of transcription factors and by culturing these cells under embryonic stem (ES)-cell conditions. The method was first described by Yamanaka using mouse fibroblasts, in which it was demonstrated that the retroviral-mediated introduction of four transcription factors — octamer-binding transcription factor-3/4, SRY-related high-mobility-group (HMG)-box protein-2, MYC and Kruppel-like factor-4 — could induce pluripotency. Both iPS cells and ES cells can be used as the pluripotent starting material for differentiated cells or tissues in regenerative medicine. There are several hurdles to be overcome before iPS cells can be considered as a potential patient-specific cell therapy, and it will be crucial to characterize the developmental potential of human iPS cell lines. As a research tool, iPS-cell technology provides opportunities to study normal development and to understand reprogramming. iPS cells can have an immediate impact as models for human diseases, including cancer.

Is-15: Keratinocyte Stem Cells Are Protected from Apoptosis

Carlo Pincelli

School of Biosciences and Biotechnologies, University of Modena e Reggio Emilia, Modena, Italy
Email: carlo.pincelli@unimore.it

Human epidermis is maintained and renewed by keratinocyte stem cells (KSC). KSC are protected from programmed cell death (apoptosis) through their anchorage with the ECM, which in turn is allowed by the presence of adhesion molecules such as integrins. Indeed, KSC are identified and characterized by highest levels of beta1 integrin that is poorly expressed in transit amplifying cells and absent in post mitotic keratinocytes. Moreover, KSC are identified by the expression of survivin, a unique member of Inhibitors of Apoptosis Protein (IAP), which is able to both inhibit apoptosis and regulate cell cycle. In human KSC, survivin expression decreases following apoptosis induced by integrin signal blockade, thus indicating that the anti-apoptotic action of survivin is involved in the survival signal mediated by integrins. In addition, survivin plays a role in keratinocyte cell cycle, under homeostatic conditions. Survivin inhibition markedly decreases keratinocyte viability and proliferation, thus reducing their ability to form colonies. Moreover, deprivation of survivin in human keratinocytes induces a cell cycle arrest at earlier times, which eventually culminates in cell apoptosis. Survivin inhibition renders human keratinocytes more susceptible to UVB-induced apoptosis. Finally, survivin over-expression in these cells protects them from apoptosis induced by UVB radiations. Altogether, these results suggest that survivin protects human KSC from apoptosis and is necessary for cell cycle progression. KSC seem to be at the origin of skin cancer, which is caused mainly by UV radiations. In human skin cancer, survivin is expressed at high levels and its intra-cellular localization changes from nuclear to cytoplasmic following treatments with therapeutic agents. It is also interesting to note that human skin cancer develops following continuous UV exposures which cause p53 mutations, and that mutated p53 clones seem to be responsible for skin carcinogenesis. Because survivin expression is negatively regulated by p53 and is restricted to the KSC population, it can play a role in cutaneous carcinogenesis.

Is-16: Keratinocyte Stem Cell Niche: The Role of Neurotrophins and Their Receptors

Carlo Pincelli

School of Biosciences and Biotechnologies, University of Modena e Reggio Emilia, Modena, Italy
Email: carlo.pincelli@unimore.it

Human epidermis is maintained and renewed by keratinocyte stem cells (KSC). KSC are surrounded by a microenvironment named “niche”, characterized both by the presence of growth factors and by the interactions between cells and the extra-cellular matrix (ECM). Neurotrophins (NT) is a family of growth factors, which control the development, maintenance and apoptotic death of neurons and also fulfill multiple regulatory functions outside the nervous system. Biological effects induced by neurotrophins strongly depend

on the patterns of neurotrophin receptor/co-receptors expression on target cells, as well as on the set of intracellular adaptor molecules that link neurotrophin signaling to distinct biochemical pathways. NT exert their functions through two transmembrane receptors, the low-affinity receptor p75 (p75NTR) which binds all NT with equal affinity and the high-affinity receptors trks. TrkA serves as a high-affinity receptor for NGF, TrkB is a high-affinity receptor for BDNF and NT-4, and TrkC is a high-affinity receptor for NT-3. All four NTs interact with the p75NTR receptor. NT form a complex network at the skin level involving most cell types and resulting in a number of autocrine and paracrine activities. Human basal keratinocytes synthesize and secrete biologically active NGF, NT-3, BDNF and NT-4. Furthermore, human keratinocytes express p75NTR, trkA and trkC. Autocrine NGF stimulates keratinocyte proliferation through its high affinity receptor TrkA, while K252, a specific inhibitor of trk phosphorylation, blocks this effect. In addition, K252 and anti-NGF antibodies induce apoptosis in human keratinocytes, indicating that autocrine NGF protects these cells from programmed cell death through its high affinity receptor. NGF is higly expressed in KSC, while it is nearly absent in transit ampligying cells (TA) and postmitotic (PM) cells. TrkA is strongly expressed in KSC and TA cells and disappears in PM cells. Blocking TrkA inhibits KSC proliferation , but does not exert any effect on TA cell proliferation. On the other hand, blocking TrkC, inhibits bot KSC and TA cell proliferation. NGF also induces the up-regulation of Bcl-2 in KSC, as compared to TA cells. p75NTR can signal on its own and mediates cell death. p75NTR is not evenly distributed in the basal keratinocyte layer, but it rather localizes in a subpopulation of basal keratinocytes. p75NTR mRNA is significantly more expressed in TA cells than in KSC. p75NTR protein is absent in KSC, while it is highly expressed in TA cells. p75NTR mediates apoptosis in human keratinocytes, while its role in KSC escaping from the niche is hypothesized. Taken together, these results indicate that NT and their receptors play a critical role within the KSC niche in human epidermis.

Is-17: The Immunological Properties of ES Cells and ES Cell-Derived Cardiomyocytes

Tomo Saric

Institute for Neurophysiology, University of Cologne, Cologne, Germany
Email: tomo.saric@uni-koeln.de

Embryonic stem (ES) cells are regarded as a very promising source of differentiated cells for tissue regeneration. ES cell-derived cardiomyocytes could functionally replace irreversibly lost cardiac tissue in various animal models of ischaemic heart disease. However, clinical application of this therapeutic approach will be hampered by immunological rejection of transplanted cells by histoincompatible recipients. To address the question of immunological properties of murine ES cell-derived cardiomyocytes we have utilized a transgenic murine ES cell line D3αPIG engineered to express GFP and antibiotic resistance specifically in ES cell-derived heart cells. This cell line enabled us to highly purify GFP-positive cardiac progenitor cells and to specifically address the question of their immunogenic properties. To this end, we have determined their immunophenotype by flow cytometry, assessed their response to the inflammatory cytokine interferon gamma, assayed their physical interaction with cytotoxic T lymphocytes and tested their susceptibility to lysis by activated NK cells and cytotoxic T cells. These studies have demonstrated that ES cell-derived cardiomyocytes constitutively express very low levels of MHC class I molecules on their cell surface, which were strongly upregulated by interferon gamma. Interestingly, the cytotoxicity experiments revealed that ES cell-derived cardiac cells were resistant to killing by poly I:C activated syngeneic and allogeneic NK cells as well as by allogeneic cytotoxic T cells. Even strong upregulation of MHC class I molecules on the surface of cardiac cells by interferon gamma did not render them sensitive to lysis by immune effector cells, indicating that transplanted ES cell-derived cardiomyocytes might be less susceptible to rejection as compared to whole organ transplants. Ongoing studies are aimed at elucidating the molecular basis of this resistance and assessing the engraftment capacity and immunogenicity of ES cell-derived cardiomyocytes in vivo upon allotransplantation.

Is-18: Functional Properties of Murine and Human ES and iPS Cell-Derived Cardiomyocytes

Tomo Saric

Institute for Neurophysiology, University of Cologne, Cologne, Germany
Email: tomo.saric@uni-koeln.de

Induced pluripotent stem (iPS) cells open new possibilities for autologous cell replacement therapies, establishment of human in vitro disease models, drug discovery and toxicology. However, before iPS cells can be used for any of these purposes the properties of their differentiated derivatives must be carefully examined. In this study we have compared functional and molecular properties of highly purified murine iPS and embryonic stem (ES) cell-derived cardiomyocytes (CM) generated from corresponding transgenic lines expressing puromycin N-acetyltransferase and green fluorescent protein under the control of a cardiospecific α-myosin heavy chain promoter. We demonstrate that murine iPS and ES cells differentiate into spontaneously beating CM at comparable efficiencies. Both iPS and ES cell-derived CM express typical cardiac transcripts and structural proteins and possess similar ultrastructural organization. Action potential recordings revealed that iPS- and ES cell-derived CM respond to adrenergic and muscarinic receptor modulation, express functional voltage-gated

sodium, calcium and potassium channels and possess comparable current densities. Comparison of global gene expression profiles of CM generated from iPS and ES cells revealed that both cell types express genes and functional categories typical for CM and cluster close to each other but are highly distant to undifferentiated ES or iPS cells as well as unpurified iPS and ES cell-derived embryoid bodies (EB). These data suggest that iPS CM obtained by lineage selection are highly similar in their structure, function and molecular characteristics to CM derived from ES cells and may represent a valuable and safe source of cells for a variety of in vitro and in vivo applications. Similar findings have also been obtained in comparative studies of microdissected human ES cell- and iPS cell-derived CM.

Is-19: Human Adult Pluripotent Stem Cells from Testis

Thomas Skutella

Anatomisches Institut der Eberhard Karls Universität Tübingen, Abteilung für Exp. Embryologie, Tissue Engineering, und Klinische Anatomie, Österbergstr. 3, D-72074 Tübingen, Germany
Email: tskutella@anatom.uni-tuebingen.de

Is-20: Germ Stem Cells

Thomas Skutella

Anatomisches Institut der Eberhard Karls Universität Tübingen, Abteilung für Exp. Embryologie, Tissue Engineering, und Klinische Anatomie, Österbergstr. 3, D-72074 Tübingen, Germany
Email: tskutella@anatom.uni-tuebingen.de

Is-21: Ex Vivo Expansion of Human Corneal Endothelial Cells

Ray Jui-Fang Tsai

Taipei Eye Center, Taiwan
Email: raytsai@ms4.hinet.net

Objective: To develop techniques to culture the human corneal endothelial cells and to identify the cultured human corneal endothelial cells maintain the property of stem cells by ex vivo expansion on amniotic membrane.
Materials and Methods: Human donor corneas were selected for studies. To understand the possibility of human corneal endothelium contains stem cells, we set up an organ culture technique for human donor cornea. Human donor cornea was cultured in culture medium with BrdU for labeling 2 weeks then chasing for another 2 weeks. BrdU labeling retention cells will be studied under immunomicroscopy. To further study the human corneal endothelial stem cells can be preserved and cultured on amniotic membrane, human corneal endothelium was obtained from the inner part of human limbal corneal rim, when the central cornea was prepared for PkP. Human corneal endothelium was cut into a small pieces and cultured on the different kind of substrate including amniotic membrane. Series passages were performed, and markers of Na/K ATPase, P63, and ABCG2 were used for immunochemistry studies. Cultured human corneal endothelial cells finally were growth on the human corneal disc.
Results: Age of human donor corneas was from 37 y/o to 70 y/o with mean 63 ± 11 y/o. The death to cultured period was from 6 days to 10 days with mean 8.4 ± 1.8 days. The cultured human corneal endothelial cells (HCEC) could be growth on the substrate with basement membrane substance. Serial passages from each HCEC were performed and results with 4.5 ± 2.3 (2 to 9 passages) had been achieved from the aged human corneas. For the study of organ culture of human donor cornea, BrdU labeling retention cells were detected only on the peripheral area of donor corneal endothelial zone, but negative over the central corneal area. To explore the corneal endothelial cells contain stem cells on the cultured system, cultured corneal endothelial cells at different culture period was studied for Na/K ATPase, ABCG2 and P63. The positive staining of ABCG2 and P63 were detected only on the earlier 2 weeks culture period but negative on the longer one month culture period. However, Na/K ATPase detected only on the longer cultured period. When HCEC was cultured on the human corneal disc, TEM demonstrated mosaic pattern of HCEC on the cornea disc
Conclusion: The ex vivo expansion of human corneal endothelial cells systems have been established. The HCEC may maintain the stem cells property on AM. Also, the cultured HCEC could be transplanted on the human corneal disc. These techniques will allow us to further study the engineering of cornea.

Is-22: The Mechanism of Ex Vivo Expansion of Limbal Stem Cells and Clinical Applications

Ray Jui-Fang Tsai

Taipei Eye Center, Taiwan
Email: raytsai@ms4.hinet.net

The successful clinical application of ex vivo expansion of limbal Stem Cells has been documented for more than 10 years. The limbal stem cells harvested in this way are effective for ocular surface reconstruction, especially in autologous transplantation. Briefly, limbal stem cells were taken from normal fellow eye of the patient and cultured in vitro for two weeks, then transplanted to the diseased eye to restore patient's vision. Therefore, to understand the mechanism of ex vivo expansion of limbal stem cells is important to further investigation of ex vivo expansion of other adult stem cells and their future clinical applications.
In order to understand the mechanism of how the hu-

man limbal stem cells can repopulate over the limbal deficient corneal surface and maintain its clarity for over 10 years, we studied the label retention cells (LRC) of limbal epithelium with BrdU staining and confirmed the existence of stem cells with P63 staining in our culture system.
The explanted culture of limbal tissue was placed on the cryo-preserved amniotic membrane in which the amniotic cells were still preserved. Culture medium with 5% patient's serum was changed every other day. 3T3 was not used as feeder layer or co-culture. PCNA staining confirmed that limbal epithelial cells proliferated better on amniotic membrane than on the plastic plate. For label retention cells (LRCs) study, BrdU was fed to limbal explanted culture continuously for one week, and then followed by chasing the BrdU and transplanted the cultured limbal stem cells with amniotic membrane into nude mice for another week. Basal layer of cultured limbal epithelium on amniotic membrane was stained positively by anti-BrdU antibody. P63 staining was also positive. These positive results confirmed that the basal cells of limbal epithelial cells on amniotic membrane were stem cells. In this system, amniotic membrane worked as niches for limbal stem cells proliferation and differentiation.
I also reviewed 54 cases that the allograft limbal stem cells had been performed during 1998 to 2000. Nineteen cases had been followed for more than 18 months were studied. There were 6 cases of chemical burn, 7 cases of OCP, 3 cases of SJ syndrome, and 3 cases with chronic inflammation. The success rate was 9/46(47.4%), including chemical burn 4/6(66.7%), OCP 3/7(42.9%), SJ syndrome 0/3 (0%), and inflammation 2/3 (66.7%). The vision improvement was 65%.
From 2001, I changed the immunosuppression regimen: prednisone 0.5mg/kg/d, cyclosporine A 2.5 mg/kg/d BID, cellcept 2g/d BID. 15 patients were included for studied. The success rate improved to 11/15(73.3%), including Chemical burn: 4/7(57.1%), OCP: 4/5(80%), SJ Syndrome 2/2(100%), and inflammation 1/1(100%).
In conclusion, in our culture system, limbal explanted culture on amniotic membrane provides a practical method for ex vivo expansion of limbal stem cells. The stem cells harvested can be transplanted to human diseased cornea and they can reserve their stem cell function for more than 10 years in autograft, but the survival rate for allograft was 50% to 70 % which depends on the immunosuppression regimen were used.

Is-23: Targeted Approaches in Stem Cell Proteomics

Dennis Van Hoof[1, 2, 3], Javier Muñoz[1], Wilma Dormeyer[1], Albert J.R. Heck[1], Christine L. Mummery[2, 4], Jeroen Krijgsveld[1, 5]

1. Biomolecular Mass Spectrometry and Proteomics Group, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Sorbonnelaan 16, 3584 CA Utrecht
2. NetherlandsDevelopmental Biology and Stem Cell Research, Hubrecht Institute, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands
3. Diabetes Center, University of California San Francisco, 513 Parnassus Avenue, San Francisco, CA 94143, USA
4. Department of Anatomy and Embryology, Leiden University Medical Center, Einthovenweg 20, 2333 ZC, The Netherlands
5. EMBL, Gene Expression Unit, Meyerhofstrasse 1, 69117, Heidelberg, Germany
Email: jeroen.krijgsveld@embl.de

Stem cells are of wide interest because of their unique biological properties, and their potential in a variety clinical application. To understand stem cell behaviour, including self renewal and differentiation under a range of experimental conditions, they should be studied using the widest possible range of molecular techniques. In this respect, proteomics takes a central position in characterizing proteins responsible for or associated with stem cell-specific function. In this presentation I will give an overview of proteomic techniques available for profiling of cells in general, and stem cells in particular. I will focus on approaches that are directed at uncovering classes of proteins that are potentially of most interest, plasma membrane proteins and phosphoproteins. I will present data showing what type of information can be obtained when dedicated sample preparation and mass spectrometric analyses are deployed.

Is-24: Articular Cartilage Repair with Autologous Bone Marrow Mesenchymal Cells in Human

Shigeyuki Wakitani

Department of Orthopaedic Surgery, Osaka City University Graduate School of Medicine, Osaka, Japan
Email: wakitani@med.osaka-cu.ac.jp

It has been reported that the mesenchymal cells in bone marrow contain progenitor cells of some mesenchymal tissues, such as bone, cartilage, fat, and muscle. Bone marrow mesenchymal cells (BMMC) are thought to be useful for reconstructing injured tissues such as bone and cartilage.
In the repair process of osteochondral defect, blood from adjacent bone marrow through the subchondral bone defect is thought to play important roles because it contains progenitor cells and growth factors. To promote the repair of osteochondral defect, cell transplantation has been investigated to make up for insufficient cells from the bone marrow. We have reported that autologous BMMC transplantation promoted the repair of full thickness articular cartilage defects in rabbit knee (J Bone Joint Surg Am 1994). This procedure is easy to perform clinically because the autologous BMMC are easy to obtain and can be culture expanded without losing their capacity for differentiation.
We transplanted autologous BMMC for the repair of full-thickness articular cartilage defects in the patellae

of a 26-year-old female and a 44-year-old male (Cell Transplantation 2004). BMMC were culture expanded, embedded in collagen gel, transplanted into the articular cartilage defect and covered with autologous periosteum. Six months after the transplantation, clinical symptoms had improved dramatically, the improvement has remained in effect (10 years and 5 months in one case, and 9 years and 7 months in the other), and both patients have been satisfied with the outcome. Histology of both patients revealed that the defect had been repaired with the fibro-cartilaginous tissue.

We transplanted BMMC to repair large articular cartilage defects in 24 knees of 24 patients with knee osteoarthritis who underwent a high tibial osteotomy (Osteoarthritis Cartilage 2002). Twelve patients received cell transplantation and the other 12 served as cell free controls. Although the clinical improvement was not significantly different 16 months after surgery, the arthroscopic and histological grading score was better in the cell-transplanted group than in the cell-free control group 8 months after the surgery. A few years ago, we investigated the clinical score for middle follow-up period (63 months), but there was no significant difference.

We transplanted BMMC into 9 full-thickness articular cartilage defects of the patello-femoral joints (including 2 kissing lesions) in the knees of three patients, a 31-year-old female, a 44-year-old and a 45-year-old male (J Tissue Eng Regen Med 2007). Six months after transplantation, the patients' clinical symptoms had improved significantly and the improvements have been maintained over the follow-up periods (17 months to 27 months). Histology of the first patient 12 months after the transplantation revealed that the defect had been repaired with the fibro-cartilaginous tissue.

We transplanted BMMC into osteochondral defects in 4 patients with osteochondritis dissecans of the elbow. The mean follow up period was 48 months (33-65). Clinical symptoms have improved significantly and they can play recreational sports. X-ray showed bone formation in subchondral area. MRI revealed that repair cartilage showed the same intensity as normal cartilage. Arthroscopy performed in 2 patients showed that articular surface was smooth like normal articular surface. Histology of the third patient 12 months after the transplantation revealed that the defect had been repaired with the fibro-cartilaginous tissue.

Autologous BMMC transplantation can be expected to become an effective method for the repair of articular cartilage defects.

Is-25: Articular Cartilage Repair with ES Cells in Rat Model.

Shigeyuki Wakitani

Department of Orthopaedic Surgery, Osaka City University Graduate School of Medicine, Osaka, Japan
Email: wakitani@med.osaka-cu.ac.jp

Since defects of articular cartilage are not completely restored, many attempts have been made to repair articular cartilage defects. Cell transplantation is one of the most promising methods but origin of the cells is one of the clinical problems in cell therapy. Embryonic stem (ES) cells are considered to be a potential tool for repairing articular cartilage defects, but so far it has been impossible to cause these cells to differentiate into chondrocytes exclusively, either in vivo or in vitro.

First, we injected ES cells into knee joints of SCID mice because we thought that joint might be chondrogenic. ES cells formed teratomas in joints. They grew bigger and bigger and subsequently they destroyed joints (Rheumatology 2003). Secondly, we transplanted ES cells into articular cartilage defects in immunosuppressed rats. ES cells (AB2.2 or CCE cells) were transplanted into articular cartilage defects in the patellar groove of immunosuppressed rats treated with cyclosporine, and histologically observed until 8 weeks after transplantation. To determine whether the repair tissue in the defect in the AB2.2 transplanted group was derived from the transplanted cells, the neomycin-resistant gene, which had been transfected into AB2.2 cells but does not exist in rat cells, was used for detection. The cells produced cartilage, resulting in repair of the defects from 4 weeks until 8 weeks after the transplantation without forming any teratomas. The neomycin-resistant gene was detected in every sample demonstrating that the repair tissue in the AB2.2 transplanted group was derived from the transplanted AB2.2 cells. The environment of osteochondral defects is chondrogenic for ES cells. ES cells may thus be a potential tool for repairing articular cartilage defects (Cell Transplantation 2004).

To explore the effect of mechanical environment on the differentiation might stimulate the differentiation of ES cells during the articular cartilage repair, we immobilized the knee joints after the transplantation of ES cells into the full-thickness articular cartilage defects in rat knees. Large mass of teratomas were formed in all the knees of immobilized group, whereas the knee defects of all the joint-free group were filled with hyaline-cartilage tissue. One mechanical factor plays an important role on differentiation of ES-cells to form hyaline cartilage tissue (J Orthop Res 2008).

Although some problems remain unsolved, ES cells are thought to be among the most promising sources for use in tissue repair because these cells are capable of both proliferation and differentiation.

Oral Presentations

Stem Cells

Os-1: Midterm Outcome of Autologous Cultivated Limbal Stem Cell Transplantation with and without Penetrating Keratoplasty

Alireza Baradaran-Rafii[1], Marzieh Ebrahimi[1], Mozhgan Rezaei Kanavi[1], Ehsan Taghi-Abadi[1], Nasser Aghdami[1], Medi Eslani[1], Pejman Bakhtiari[1], Bahram Einollahi[1], Mohammad-Ali Javadi[1], Hossein Baharvand[1, 2]

1. Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cells Biology and Technology, ACECR, Tehran, Iran
2. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran
Email: baharvand@royaninstitute.org

Objective: To report the midterm outcomes of autologous limbal stem cell transplantation cultivated on amniotic membrane (AM) with or without subsequent penetrating keratoplasty (PKP) in patients with total unilateral limbal stem cell deficiency (LSCD).
Materials and Methods: Eight eyes of 8 consecutive patients with unilateral total LSCD underwent autologous limbal stem cell transplantation cultivated on AM. Four eyes underwent subsequent optical PKP. Main outcome measures were corneal vascularization and transparency.
Results: The patients were followed for 34.0 ± 13.5 months (6 to 48). Seven cases had a stable corneal epithelium with marked decrease in opacification and vascularization. Progressive sectorial conjunctivalization was evident in all cases with subsequent PKP at the last follow-up. Primary failure was observed in one case due to exposure.
Conclusion: Transplantation of autologous stem cells cultivated on AM with or without subsequent PKP seems to be an effective way for visual rehabilitation in total LSCD. More work with more cases and longer follow up are needed to optimize this procedure in order to provide and maintain an adequate supply of limbal stem cells in these patients.

Keywords: Cornea, Limbal Stem Cell Deficiency, Cultured Cells, Stem Cell Transplantation, Penetrating Keratoplasty

Os-2: Quantitative Proteomics of Human Embryonic Stem Cell Differentiation

Mahdyie Jadaliha[1,2], Hyoung-Joo Lee[3], Mohammad Pakzad[4], Seul-Ki Jeong[3], Sang-Yun Cho[3], Hossein Baharvand[4, 5], Young-Ki Paik[3], Ghasem Hosseini Salekdeh[1, 6]

1. Department of Molecular Systems Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Biotechnology, College of Science, University of Tehran, Tehran, Iran
3. Department of Biochemistry, Yonsei Proteome Research Center and Biomedical Proteome Research Center, Yonsei University, Sudaemoon-Ku, Seoul, Korea
4. Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
5. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran
6. Department of Physiology and Proteomics, Agricultural Biotechnology Research Institute of Iran, Karaj, Iran

Gene expression analyses of the embryonic stem cells (ESCs) will lead to uncover or further define signaling pathways and molecular mechanisms involved in the maintenance of self-renewal and pluripotency. We performed a mass spectrometry-based quantitative analysis using 8-plex iTRAQ labeling to identify differentially expressed proteins in human ESC line, Royan H5, during spontaneous differentiation by embryoid body formation in different stages (ESC, EBs 6, 12 and 20 days after differentiation initiation). Evaluated by strict criteria, we successfully identified 391 proteins present in more than three replicates, including different proteins involved in cell proliferation, cell apoptosis, transcription, translation, mRNA processing and protein folding. The differential expression pattern was significantly observed among 190 proteins such as down-regulation of important proteins like Lin- 28 homolog A, BAF155, and Galectin-1 which are taking part in regulating pluripotency and proliferation in ESCs as well as up-regulation of various proteins such as Ezrin, Thrombospondin-1, Serpin B9, Protein DJ-1 and Cathepsin B. The further analysis is in progress.

Keywords: Proteomics, Human Embryonic Stem Cell, Gene Expression, iTRAQ, 8-Plex

Os-3: OCT4 Spliced Variants Are Differentially Expressed in Human Pluripotent and Nonpluripotent Cells

Kenneth P McNatty

Mowla S.J[1]*, Atlasi Y[1], Ziaee S.A.M[2], Gokhale P.J[3], Andrews P.W[3]

1. Department of Genetics, Faculty of Basic Sciences, Tarbiat Modares University, Tehran, Iran
2. Urology and Nephrology Research Center, Shahid Beheshti University of Medical Sciences, Tehran ,Iran
3. Centre for Stem Cell Biology, Department, Sheffield, United Kingdom
Email: sjmowla@yahoo.com

Objective: OCT4 is a master regulator of self-renewal

in embryonicstem cells and can potentially encode two spliced variants, designated OCT4A and OCT4B.
Materials and Methods: We have examined the expression pattern of these OCT4 isoforms in various human pluripotent and nonpluripotent cells.
Results: Our data revealed that whereas OCT4A expression is restricted to embryonic stem (ES) and embryonal carcinoma (EC) cells, OCT4B can be detected in various nonpluripotent cell types. Furthermore, we detected a novel OCT4 spliced variant, designated OCT4B1, that is expressed primarily in human ES and EC cells and is downregulated following their differentiation. We also found a significantly higher level of OCT4B1 expression in stage-specific embryonic antigen-3 (SSEA3)(+) compared with SSEA3(+) subpopulations of cultured ES cells.
Conclusion: Taken together, our data demonstrated a distinctive expression pattern for OCT4 spliced variants in different cell types and highlight the necessity of defining the type of OCT4 when addressing the expression of this gene in different human cells.

Keywords: OCT4, Embryonic Stem Cells, Cancer, Spliced Variants, OCT4B1

Os-4: An Efficient and Easy-to-Use Protocol for Cryopreservation of Feeder Free Human Pluripotent Stem Cells

Seyedeh Nafiseh Hassani[1], Sepideh Mollamohammadi[1], Adeleh Taei[1], Mohammad Pakzad[1], Mehdi Totonchi[1, 2], Ali Seifinejad[1], Najmehsadat Masoudi[2], and Hossein Baharvand[1, 3*]

1. Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Genetics, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran
Email: baharvand@royaninstitute.org

Optimization and development of more defined culture methods for human embryonic and induced pluripotent stem cells (hESCs and hiPSCs) will provide an invaluable contribution to the field of regenerative medicine. However, one problem is that hESCs and hiPSCs are vulnerable to apoptosis upon cellular detachment and dissociation and therefore have a low plating efficiency upon passaging. Here, we report the effective freezing/thawing of single dissociated hESCs and hiPSCs in a feeder-free culture in the presence of ROCK inhibitor Y-27632. Our results show that the addition of Y-27632 to medium of hESCs and hiPSCs increases the survival of single dissociated hESCs and hiPSCs in feeder-free culture. The cloning efficiency of hiPSCs and hESCs improves when ROCK inhibitor is added both in Matrigel and in medium in comparison with conventional addition to medium alone. Under these treatments hESCs and hiPSCs retain typical morphology, stable karyotype, expression of pluripotency markers and the potential to differentiate into derivatives of all three germ layers after long-term culture. Therefore, we believe this method will be useful for current and future applications of these pluripotent stem cells.

Os-5: Mathematical Modeling of Protein-Protein Interaction Network Using Information Theory Approach

Leila Pirhaji[1, 2], Lars Malmstroem[3], Ghasem Hosseini Salekdeh[2]

1. Department of Biotechnology, College of Science, University of Tehran, Tehran, Iran
2. Department of Molecular Systems Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Institute of Molecular Systems Biology, ETH Zurich, Zurich, Switzerland

Understanding and modeling of cellular processes depend on comprehensive information of protein networks. Large-scale affinity purification coupled with mass spectrometry (AP-MS) provided comprehensive data for the analysis of protein complexes. In large-scale AP-MS experiment, there are many different conditions in which different proteins are tagged, and in each pull-down there is high number of proteins which include a lot of contaminants. So dealing with this large amount of data to infer a reliable protein-protein interaction network is an essential task. Here, we propose a new algorithm which uses the concept of information theory for analyzing the parallel proteomic data. Information-theoretic methods use mutual information, which is an information-theoretic measure of dependency. Mutual information is being used for calculating the association score of each protein interaction based on measuring the similarity of protein profiles among different pull-downs. So with this algorithm we will be able to infer protein-protein interaction network with weighted edges using quantitative mass spectrometry, in which the weight of each interaction indicate the probability of the occurrence of that interaction.

Os-6: Proteomic Profiling of the Central Nervous System in Murine Experimental Autoimmune Encephalomyelitis before and after Treatment with Mouse Embryonic Stem Cell-Drived Neural Precursor Cells

Abolhassan Shahzadeh Fazeli[1, 2, 3], Davood Nasrabadi[1], Mohammad Hossein Sanati[3], Ali reza Pouya[1], Hossein Baharvand[1, 4], Ghasem Hosseini Salekdeh[1, 5]

1. Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology,

ACECR, Tehran, Iran
2. Department of Genetics, Royan institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. National institute for Genetic Engineering and Biotechnology, Tehran, Iran
4. Department of Developmental Biology, university of Science and culture, ACECR, Tehran, Iran
5. Department of Physiology and Proteomics, Agricultural Biotechnology Research Institute of Iran, Karaj, Iran

Proteomics approach to investigate protein changes in central nervous system (CNS) of mouse Experimental Autoimmune Encephalomyelitis (EAE) before and after treatment with mouse embryonic stem cell-derived neural precursor cells (ESC-NPs) in order to identify candidate mechanisms of damage in lesions of multiple sclerosis (MS). EAE induced by myelin oligodendrocyte glycoprotein. Intravenous injection of mouse ESC-NPs in EAE at score 3. Protein expression profiles in CNS between healthy, and clinical score 3 of EAE and treatment EAE were studied using two dimensional electrophoresis based proteomics approach coupled with MALDI TOF/TOF mass spectrometry. Clinical improvement was observed in EAE after the ESC treatment. Expression level of 30 proteins (out of 72 differentially expressed in EAE) were dramatically changed. The level of these 30 proteins was statistically under the differentiation border compare to the healthy control. Identified proteins supported the hypothesis that ESC help to decrease inflammation and increase remyelination in CNS of EAE. The exact function of these proteins and their involvement in the ESC-NPs treatment mechanism is under investigation.

Keywords: Proteomics, EAE, MS, ESC

Os-7: A Complexomic Study of Human Embryonic Stem Cells and Human Embryonic Carcinoma Cells

Faezeh Shekari[1], Adele Taei[2], Hossein Baharvand[2, 3], Ghasem Hosseini Salekdeh[1, 4]

1. Department of Molecular Systems Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran
4. Department of Physiology and Proteomics, Agricultural Biotechnology Research Institute of Iran, Karaj, Iran

Since, a protein complex can be considered as a minimal biological structure of a cellular compartment , complexomic study of human embryonic stem cells and human embryonic carcinoma cell has been done for the first time via Blue native polyacrylamide gel electrophoresis (BN-PAGE) following by denaturing SDS-PAGE. Membrane and cytosolic fractions of hESC(Royan H5) and hECC(N-Tra2) has been prepared and the reliability of the fractionation has been confirmed by western blotting with utilization of membrane and cytosolic markers. More than 70 membrane proteins and about 100 cytosolic ones from BN-PAGE proteome maps were identified using MALDI TOF/TOF. Different possible protein complexes were validated by computational prediction of possible protein-protein interactions according to STRING databank and confirmed protein complexes based on the molecular weight of complex estimated by BN PAGE and STRING results introduced as Cytosolic Protein Complex (CPC) and Membrane Protein Complex (MPC) and also homooligomers. Database search revealed many attractive results about protein complexes and active metabolic pathways in both hESC and hECC.

Os-8: Dental Stem Cells-Based Tissue Regeneration in a Large Animal Model

Yi Liu[1]*, Songlin Wang[1], Songtao Shi[2], Ying Zheng[1], Dianji Fang[1], George T.J. Huang[3], Chunmei Zhang[1]

1. Laboratory for Gene Therapy & Tooth Regeneration, Capital Medical University School of Stomatology, Beijing, P. R. China
2. The Center for Craniofacial Molecular Biology, University of Southern California School of Dentistry, Los Angeles, USA
3. College of Dental Surgery, Dental School, University of Maryland, Baltimore, USA
Email: lililiuyi@163.com

Objective: The dental tissues contain a variety of stem cells, including dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs), stem cells from the apical papilla (SCAP). These dental tissues-related stem cells possess high proliferation and differentiation capacities, and can be acquired easily from useless teeth, such as exfoliated deciduous teeth (SHED), extracted impacted third molars or orthodontic extracted teeth (DPSCs, PDLSCs and SCAP). The purpose of our studies is to perform oral and craniofacial tissue regeneration using these dental tissues-related mesenchymal stem cells at pre-clinical level in a large animal model (swine).
Materials and Methods: Dental related stem cells were obtained from extracted teeth of the miniature pigs and then expanded ex vivo to enrich cell numbers. The characteristics and differentiation abilities of these stem cells were analyzed. Then, we utilized these stem cells to treat created periodontal lesions, to regenerate bioroots and to repair critical size bone defects in miniature pigs. (1) A typical periodontitis animal model was developed on miniature pig's first molar. PDLSCs were utilized to treat periodontal defect lesion and regenerate

the new periodontal tissues. (2) Using a minipig model, autologous SCAP and PDLSCs were loaded onto HA/TCP and gelfoam scaffolds, respectively, and implanted into sockets of the lower jaw. Three months later, the bioroot was exposed, and a porcelain crown was inserted. (3) Autologous SHED were used to repair critical-size mandibular bone defects in minipig. (4) PDLSCs and bone marrow mesenchymal stem cells (BMMSC) were used to reconstruct orofacial tissues for changing orofacial appearance.

Results: Dental tissues related stem cells could be isolated successfully from miniature pig. A subset of these cells retained expression of early-stage markers of stem cells and had the abilities of single colon forming, high proliferation and differentiation. (1) PDLSCs appeared to have an excellent capacity to form bone, cementum, and periodontal ligament. The GFP-labeled cells were present in newly formed periodontal bones and had differentiated to osteoblasts, suggesting that transplanted PDLSCs had contributed to periodontal tissue regeneration in vivo, leading to a favorable treatment for periodontitis. (2) The structures of bioroot developed by SCAP and PDLSCs were still different from a natural root in a random manner. Nevertheless, the bioroot was encircled with periodontal ligament tissue and appears to have a natural relationship with the surrounding bone, and the mechanical strength of bioroot was about 70% of normal tooth root. Although there were many challenges, the approach was relatively a quick way of creating a root onto which an artificial crown could be installed. (3) SHED (SPDs) were capable of regenerating critical-size defects in the orofacial bone, and might potentially serve as an alternative stem-cell-based approach in the reconstruction of alveolar and orofacial bone defects. (4) BMMSCs could change the orofacial appearance by extending body bone tissues in minipig and subcutaneous transplantation of PDLSCs could form substantial amounts of collagen fibers and improve facial wrinkles in mouse.

Conclusion: These studies demonstrate dental tissues-related stem cells are hidden treasures and provide promising potential application for tissue engineering to oral and craniofacial plastic surgery and diseases therapy.

Keywords: Dental Relative Stem Cell, Craniofacial Disease, Therapy, Tissue Engineering, Regeneration

Os-9: Investigating Functional Consequences of miRNA Expression During Somatic Cell Reprogramming

Mohammadsharif Tabebordbar[2, 1], Stuart Levine[1], Ghasem Hosseini Salekdeh[2], Laurie A. Boyer[1]

1. Massachusetts Institute of Technology, Department of Biology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA

2. Department of Molecular Systems Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

The recent generation of induced pluripotent stem cells (iPSCs) from somatic cells provides an invaluable resource for drug or toxicology screening, medical research, and patient-specific cell therapy. However, the mechanisms underlying reprogramming still remain largely unknown. Reprogramming is a gradual process in which a small fraction of transduced cells undergo global changes in gene expression profiles, histone modification and DNA methylation patterns, while the majority of cells do not fully reprogram and remain in a partially reprogrammed state. MicroRNAs which are ~22 nucleotide single-stranded RNA molecules, have emerged as important regulators of gene expression and also appear to converge with the core regulatory circuitry controlling self-renewal and pluripotency in ES cells. To investigate the role of miRNAs in the process of reprogramming, we defined the set of miRNAs expressed during different stages of mouse embryonic fibroblast (MEF) reprogramming by deep sequencing. We found a subset of miRNAs that are differentially expressed in MEF, intermediate cells and iPSCs. Significant differences in the expression level of some miRNAs in different stages of reprogramming suggest that these molecules, along with other regulators of gene expression, might have a role in de-differentioan of somatic cells. We are currently testing the role of a selective set of miRNAs in reprogramming by a variety of methods.

Poster Presentations

Stem Cells

Ps-1: Leptin and Leptin Receptor mRNA in the Bovine Testis

Abavisani A[1]*, Shayan P[2], Baghbanzadeh A[2], Tajik P[2]

1. Physiology Section, Department of Basic Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
Email: abavi1355@yahoo.com

Leptin, hormonal product of the ob gene, is known to regulate food intake, energy metabolism and reproductive functions in mammals. The mechanism by which leptin affect male reproductive system, in contrast to its well proven effects in female fertility, has been a matter of debate. Expression of leptin and its receptor in some reproductive organs suggests that leptin has both endocrine and paracrine/autocrine effects on reproduction. Various evidences have pointed to a direct role of leptin in the control of rodent testicular function such as steroidogenesis and spermatogenesis. Thus, detection of leptin and leptin receptor mRNA in bovine testis will be the first crucial step to an understanding of its paracrine/autocrine effect on testes in cattle.
In the present study, we showed leptin mRNA as well as its functional receptor (Ob-Rb) mRNA in whole testis of Holstein cattle using reverse transcription and polymerase chain reaction analysis. To confirm the first results, RT-PCR products were amplified with Nested PCR using inner leptin primer pairs designed on different exons. Based on our results, although we did not determine the exact cell source of leptin in testis, it suggests that besides its primary actions at the hypothalamic-pituitary level, leptin can has a direct role in testicular physiology using autocrine and/or paracrine mechanisms.

Keywords: Cattle, Leptin, Leptin Receptor, Testis

Ps-2: Efficient Replacing of Fetal Bovine Serum with Platelet Lysate during Propagation and Differentiation of Human Bone Marrow Derived Mesenchymal Stem Cells to Functional Hepatocytes-like Cells

Allameh A[1]*, Kazemnezhad S[2, 3], Soleimani M[2], Gharehbaghian A[3], Amirizadeh N[3]

1. Department of Clinical Biochemistry, Faculty of Medical Science, Tarbiat Modares University,Tehran, Iran
2. Avicena Research Institute, Shahid Beheshti University, Tehran, Iran
3. Department of Hematology, Faculty of Medical Science, Tarbiat Modares University, Tehran, Iran
3. Research Centre of Blood Transfusion Organization, Tehran, Iran
Email: kazemnejad_s@yahoo.com

Objective: The studies in stem cell biology have shown that mesenchymal stem cells (MSCs) can differentiate into hepatocytes. Traditionally, fetal bovine serum (FBS) has been used for expansion and differentiation of MSCs to different lineages. However, certain risks associated with the use of FBS have stimulated a search for alternatives. The aim of this study was to find out substitution effect of FBS with human platelet releasate (HPR) as a major growth factor source during expansion and differentiation of human bone marrow derived MSCs into hepatocytes.
Materials and Methods: For this purpose, parallel experiments were carried out using either the conventional culture media (containing FBS) or HPR- supplemented media.
Results: MTT assay showed that HPR was more efficient than FBS in supporting hBMSC outgrowth. The proliferation rate of MSC in presence of HPR (derived from 109 platelets/ml) was about 3-fold greater than that of FBS. Despite these differences in MTT value, hBMSCs driven HPR and hBMSCs driven FBS did not differ in terms of gross morphology, immunophenotype and osteogenic differentiation potential. The evidences presented in this paper show that the hepatic differentiation of hBMSCs, was successfully completed in the media enriched with HPR. Immunoreactivity of cells with monoclonal antibodies against hepatocyte markers such as albumin and alpha-fetoprotein (AFP) as judged by immunofluorescent staining was even more positive in hepatocytes differentiated in presence of HPR as compared to that of FBS ($p<0.05$). Moreover, the mRNA expression of albumin, AFP, cytokeratin-18, cytokeratin-19 and cytochrome-P 450 in hBMSCs derived cells in HPR attest to supporting role of HPR in hepatic differentiation media. These findings were further confirmed with greater urea production (~2-fold) in the culture media of cells differentiated under HPR compared to that in FBS ($p<0.01$).
Conclusion: Overall results presented here show that the efficient propagation and differentiation of human MSCs with HPR not only eliminate the risk of bovine pathogen contamination and xenoimmunization, this would represent a major novel step towards safe stem cell therapy and liver tissue engineering.

Keywords: Mesenchymal Stem Cell, Hepatocyte, Differentiation, Platelet

Ps-3: The Effects of Adult Neural Stem Cell Transplantation by Lumbar Puncture in the Repair of Injured Brain by Transient Cerebral Ischemia in Rats

Aliaghaei A[2]*, Seyyed Jaafari S.S[2], Nematollahi S.N[2],

Sheibani V[1], Asadi M[1]

1. Kerman Neurosciences Center, Kerman, Iran
2. Schol of Medical Science, Kerman, Iran
Email: aghaei60@gmail.com

Objective: Ischemia is the third cause of mortality in many countries, and many people suffer from the following disabilities. Ischemia causes a series of changes in the brain parenchyma leading to induction of cell death. Stem cells have the ability of self renewal therefore are a good source for cell therapy in the ischemia. The stem cells, based on their origin, are classified into embryonic and adult types. Adult stem cells exist in all tissues of every creature and are a replacement for the lost cells in related tissue. The subventricular zone of anterior horn of the lateral ventricle and dentate gyrus in the hippocampus are the main areas that neural stem cell are stored in the brain.
Materials and Methods: In this study the neural stem cells obtained from subventicular zone of adult rats were cultured in DMEM/F12 culture medium with N2 supplement in the presence of EFG and FGF2 mitogenic factors. To induct the ischemia, the animals were anesthetized and after dissection the middle cerebral artery was occluded for one hour. Three days after induction of the ischemia the neural stem cells which were labeled with PKH26 were injected into animals by lumbar puncture at the level of L5-S1. The motor behavioral functional recovery in 1st, 7th, 14th, 21th, and 28th days after induction of the ischemia was examined by Rotarod test. The presence of neural stem cells in the brain tissue of the animals was examined by immunohistochemistery and immunohistofleurscent methods after Rotarod test.
Results: The results showed that the motor behavioral functional recovery in ischemic group of animals that received neural stem cells by lumbar puncture was better than ischemic groups of animals that did not receive neural stem cells. The labeled cells with PKH26 immunofluorescence marker were observed in the ischemic area of the brain tissue sections in lumbar puncture groups stained by the immunohistochemistry staining technique. Alkaline phospahatase test and immunohistochemistery staining technique demonstrated a gathering of neural stem cells in the lateral ventricle that were injected into animals by lumbar puncture method. It was also observed that a number of cells migrated from lateral ventricle through the ependymal layer to the adjacent brain parenchyma. These cells expressed neuronal markers (NSC and β-tubulin3) and astrocytic markers (GFAP and S100).
Conclusion: It is concluded that the neural stem cells injected lumbar puncture methods were able to migrate to the injured area by the ischemia and differentiate in to neural phenotypic cells. These differentiated cells caused the recovery of the motor function after the induction ischemia..

Keywords: Neural Stem Cells, Lumbar Puncture, Cerebral Ischemia

Ps-4: Dehydroepiandrosterone Stimulates Neurogenesis in Mouse Embryonic Carcinoma and Human Embryonic Stem Cell-Derived Neural Progenitors and Induces Dopaminergic Neurons

Azizi H[1]*, Zare Mehrjerdy N[1], Shahbazy E[1], Baharvand H[1, 2], Kiani S[1]

1. Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran
Email: hosseinazizi58@yahoo.com

Objective: To evaluate the effect of Dehydroepiandrosterone (DHEA) as a neurosteroid on the rate of neurogrnesis, neural survival and proliferation of pluripotent stem cells-derived neurons, we have added DHEA on mouse P19 embryonal carcinoma cells (ECC) and human embryonic stem cells (ESCs)-neural progenitors (NPs).
Materials and Methods: Flow cytometric, quantitative RT-PCR and Immunocytochemistry analysis showed the percentage of tyrosin hydroxylase (TH), Nurr1, Nestin, BrdU and Tuj1 positive cells. The expression of neuronal specific genes such as Mash1, Pax6, Tuj1,EsR, TH, was also detected by RT-PCR analysis, and also apoptosis was detect to annexinV assay.
Results: In ECC-derived NPs, flow cytometric analysis of Nestin- and Tuj1-positive cells revealed that the percentages of these cells were increased significantly for the markers following DHEA treatment of the cells. Moreover, the percentages of tyrosin hydroxylase (TH)-positive cells, the marker of dopaminergic neurons significantly increased in presence of DHEA.The BrdU incorporation and Estrogen receptor (EsR) found to have increased after DHEA induction. Moreover, the apoptosis was significantly decreased after DHEA treatment. DHEA effect also confirmed on human ESCs-derived NPs by enhancement of Tuj1- and TH-immunofluorescent positive cells and TH and Nurr1 transcripts as detected by quantitative RT-PCR.
Conclusion: In conclusion, these results have presented evidence DHEA is able to induce neurogenesis in mouse ECC and human ESC cells-derived NPs. This observation is related to the division of NPs and the reduction of apoptosis. Moreover, DHEA has dopaminerdic potential in the both species cells. This provides a better insight into the differentiation and maintenance of neural cells and treatment of a wide variety of neurological diseases such as Alzheimer and Parkinson by stem cells.

Keywords: Embryonal Carcinoma Cells, Dehydroepiandrosterone, Human Embryonic Stem Cells, Neural Differentiation

Ps-5: Amniotic Fluid Versus Bone Marrow Derived

Mesenchymal Stem Cells: Proliferation, Bone Differentiation and Senescence

Baghaban Eslaminejad M.R, Aghdami N, Jahangir Sh*

Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: sh.jahangir@gmail.com

Objective: Mesenchymal stem cvells (MSCs) have been known as an appropriate candidate for cell replacement therapy of tissue defects including those associated with skeletal system. These cells so far have been isolated from several tissue sources as bone marrow(BM) and amniotic fluid(AF), but little is known about the differences existed between the cells from these sources. This subject was considered in the present investigation.
Materials and Methods: Amniotic fluid and bone marrow cells were collected from NMRI mice and culture expanded through several passages. To ensure the MSCs nature, isolated cells were differentiated among bone, cartilage and adipose cell lineages. Moreover, the cells from two sources were compared to each other in terms of the proliferation rate, the potential of bone differentiation and the senescence in culture by calculating population doubling time (PDT), quantification of culture mineralization and determining the culture beta-galactosidase positive cells respectively.
Results: Culture-expanded cells from either source appeared to be able to generate bone, cartilage and adipose cells upon providing with appropriate inductive microenvironment hence being MSCs in nature. The PDT value for AF MSCs was significantly less than that for BM MSCs (92.6 ± 13.9 versus168 ± 40 hours, $p<0.05$) indicating their more rapid proliferation in culture. According to our data, there was no significant difference regarding the mineralization of osteogenic cultures prepared from either cell, although the value of AF cells tended to be slightly higher than BM cells. With respect to the cell senescence, passaged-7 bone marrow cells possessed statistically more beta galactosidase positive cells (57 ± 6) than amniotic cells (39 ± 4). This implies that BM MSCs reach senescence earlier than their AF counterparts.
Conclusion: Collectively the results indicate some advantageous characteristics of AF derived MSCs over bone marrow MSCs, suggesting that AF-MSCs would be an appropriate candidate for stem cell applications.

Keywords: Mesenchymal Stem Cells, Amniotic Fluid, Bone Marrowe, Differentiation, Proliferation, Ageing

Ps-6: Successful Vitrification of Rat Bone Marrow Derived Mesenchymal Stem Cells

Bahadori M.H[1, 5]*, Soltani B[2, 5], Mirzajani E[3, 5], Babaee P[4, 5] , Ansar M.M[1, 5]

1. Aanatomy Department, Faculty of Medicine, Guilan University of Medical Sciences, Rasht, Iran
2. Pharmacology Department, Faculty of Medicine, Guilan University of Medical Sciences, Rasht, Iran
3. Biochemistry Department, Faculty of Medicine, Guilan University of Medical Sciences, Rasht, Iran
4. Physiology Department, Faculty of Medicine, Guilan University of Medical Sciences, Rasht, Iran
5. Cellular and Molecular Research Center, Faculty of Medicine, Guilan University of Medical Sciences, Rasht, Iran
Email: bahadori@mail.com

Objective: Mesenchymal stem cells (MSCs) are obtained from a variety of sources, mainly bone marrow. These cells have great likely for clinical research due to their potential to regenerate tissue. A cryopreservation procedure for MSCs is required because these cells cannot stay alive for long periods in culture. The aim of this study was to determine whether vitrification is a useful freezing method for storage of MSCs.
Materials and Methods: Mesenchymal stem cells were isolated from rat bone marrow based on their capacity to adhere to plastic culture surfaces. MSCs were cryopreserved using both vitrification method and OPS vitrification and stored at -196 °C in liquid nitrogen with EFS as cryoprotectant for two months. The morphology and viability of thawed MSCs were evaluated by Trypan Blue staining. Furthermore, pre and post cryopreserved MSCs induced to osteocyte and adipocyte with corresponding osteogenic and adipogenic medium for three weeks and alizarine red S and oil red- O staining were done.
Results: We were able to obtain homogeneous plastic adherent cells from the mononuclear cell fractions of bone marrow using our culture conditions. After thawing, the viability rates was 81.33% ± 6.83 for vitrification method and 80.83% ± 6.4 for OPS vitrification, while the values with the before vitrification control group were 88.16% ± 6.3 (Mean ± SD, n = 6). Post-cryopreserved cells from both of vitrification method and OPS vitrification also had similar cellular morphology and colony-formation indistinguishable from the non-vitrified fresh MSCs. In addition the resuscitated cells cultured in induction medium consisting of 100 nM dexamethasone, 10 mM ß-glycerol phosphate and 50 µM ascorbic acid-2-phosphate, showed osteogenesis, and mineral production and deposition was detectable after 21 days by alizarine red S staining. Moreover, applying adipogenic differentiation condition, pre and post cryopreserved cells differentiated into adipocyte by 5µg/ml insulin, 1 µM dexamethasone, 100 nM Indomethacine, 0.5 mM methylisobutylxanthine and lipid vacuole accumulation was stained by oil red O.
Conclusion: This study indicates that vitrification is a reliable and effective method for cryopreservation of MSCs.

Keywords: Mesenchymal Stem Cells, Cryopreserva-

tion, Differentiation, Vitrification

Ps-7: Effects of Vitreous Humour on Growth and Differentiation of the Rat Mesenchymal Stem Cells (rMSCs) and Human NTERA2 Cells

Bahrami A.R*, Moghadam Matin M, Edalatmanesh M.A, Jian Bagherpoor A

Ferdowsi University of Mashhad, Mashhad, Iran
Email: ajbagherpoor@yahoo.com

Objective: A natural source of Hyaluronic Acid (HA), rabbit vitreous humour (VH), was previously shown to promote wound repair in model animals. In search for its possible mechanisms, VH extract was tested on the cultured stem cells.
Materials and Methods: Vitreous humour treatment of the cultured stem cells, RT-PCR, Flow cytometry analysis
Results: The cellular and molecular markers (A2B5, Oct4, Sox2) changes showed that VH and possibly HA interferes with differentiating effect of Retinoic Acid.
Conclusion: This reagent may affect cell proliferation and tissue regeneration by inhibition of cell differentiation.

Keywords: Differentiation, Proliferation, Stem Cells, Vitreous Humour

Ps-8: Cardiac Differentiation of P19CL6 Cells by Oxytocin

Fathi F[1]*, Mowla S.J[2], Kermani A.J[2], Asahara T[3], Murasawa S[3], Hesagawa S[3]

1. KDRC, Anatomy Department, Kurdistan University of Medical Sciences, Sanandaj, Iran
2. Genetics Department, Faculty of Basic Sciences, Tarbiat Modares University, Tehran, Iran
3. Group of Vascular Regeneration Research, Riken Center for Developmental Biology, Kobe, Japan
Email: farfath@yahoo.com

Objective: It has been reported that P19 embryonal carcinoma (EC) cells differentiate into beating cardiomyocytes under the action of oxytocin (OT). It has been suggested that dimethylsulfoxide (DMSO) acts via the oxytocin/oxytocin receptor pathway because an oxytocin receptor antagonist not only blocks oxytocin-induced cardiomyocyte differentiation, but also blocks DMSO-induced differentiation. In this study, the differentiation ability of OT was tested using P19CL6 cells
Materials and Methods: P19CL6 cells were cultured as a confluent monolayer and aggregated cells. OT was then added to culture media as an inducing agent. The cells treated with 1% DMSO were used as a positive control group. Differentiated cells were evaluated morphologically and immunocytochemically, as well as by RT-PCR. In addition, a stable line of green fluorescent protein (GFP)-expressing P19CL6 cells were differentiated into beating cardiomyocytes by OT.
Results: Aggregated P19CL6 cells could be differentiated into cardiomyocytes, whereas monolayer cells could not differentiate and express specific cardiac muscle marker genes. In the control group, both aggregates and monolayer cells could be differentiated into cardiomyocytes by DMSO. In addition, GFP-expressing P19CL6 cells differentiated efficiently into beating cardiomyocytes when treated with OT. The results of all evaluations confirmed that the differentiated cells were cardiomyocytes.
Conclusion: We concluded that embryoid body formation (cell aggregation) is necessary for the differentiation of P19CL6 cells into cardiomyocytes when using OT as an inducer agent. Furthermore, because of the high rate of differentiation efficiency, GFP-expressing cardiomyocytes derived from P19CL6 cells have the potential to be used for regenerative therapies in experimental models.

Keywords: Cardiomyocytes, Cell Differentiation, Embryoid Body Formation, Oxytocin, P19CL6 Cells

Ps-9: *In Vitro* Differentiation of Rat Mesenchymal Stem Cells into Hepatocyte

Ghadarjani Sh[1, 2]*, Soltani B[1], Bahadori M.H[1], Ahmadi M[1]

1. Cellular and Molecular Research Center, GUMS, Rasht, Iran
2. Talented Students Office, GUMS, Rasht, Iran
Email: shervin.gh@gmail.com

Objective: Mesenchymal stem cells are pluripotent cells that are able to differentiate into several lines of adult cells. The differentiated cells are as like as adult cells in the body of the mammalian which the stem cells where taken from with the same function and thereby could be used in either research or therapeutic studies.
Materials and Methods: Mesenchymal stem cells extracted from Rat's bone marrow was previously proven to be stem cells by the adhesive property to the floor of the dish and also the ability to be differentiated into fat and bone cells where fat cells were colored by Oil Red and the bone cells by Alizarin Red either. These mesenchymal stem cells were exposed to Fibroblast Growth Factor and Hepatocyte Growth Factor in vitro and the result differentiated cells directed to be colored by Periodic Acid Shift (PAS).
Results: The differentiated cells could truly be colored by PAS and this was a clue to know these differentiated cells as hepatocytes.
Conclusion: The Hepatocyte could be another target for mesenchymal stem cells to be differentiated like many other cell lines in vitro.

Keywords: Mesenchymal Stem Cells, Differentiation, Hepatocyte

Ps-10: Construction of a Plasmid Containing Attachment Site (attB) Sequence Related to ΦC31 Integrase Next to GFP cDNA with Murine Oct-4 Promoter

Ghorbani Jarghouyeh R[1]*, Khazaie Y[2], Dormiani K[2], Forouzanfar M[2], Karbalaie KH[4], Karamali F[4], Nematollahi M[4], Ghaedi K[2, 3], Nasr Esfahani M.H[2], Rabbani M[1]

1. Biotechnology Department, Faculty of Modern Sciences and Technology, Isfahan, Iran
2. Department of Reproduction and Development, Royan Institute for Animal Biotechnology, ACECR, Isfahan, Iran
3. Biology Department, Faculty of Science, Isfahan University, Isfahan, Iran
4. Department of Cell and Molecular Biology, Royan Institute for Animal Biotechnology, ACECR, Isfahan, Iran
Email: id_ghorbani@yahoo.com

Objective: In order to study the Oct-4 regulation and its function in the differentiation process, we have designated to clone its related promoter upstream of EGFP as a gene marker.Oct-4 is a transcription factor of the POU family. This protein is critically involved in the self-renewal of differentiated embryonic stem cells and also is a transcription factor used to create induced pluripotent stem cells, demonstrating its capacity to induce an embryonic stem cell-like state. As such, it is used as a marker for undifferentiated cells.
Materials and Methods: OCT4 promoter was cloned using murine genome and placed near to EGFP gene as a marker gene for further analysis. Moreover the recognition sites for integrase were put near to the constructed sequences for efficient insertion into the target genomes.
Results: Sequence analysis and transfection data confirmed that accuracy of cloned promoter which EGFP showed a clear fluoresceny upon tranfection into the mouse embryoninc stem cells.
Conclusion: Co-transfecting of this plasmid and a vector expressing integrase cDNA, into the murine stem cell line (Royan B1), we obtained numerous transformant cell colonies expressing EGFP under regulation of this promoter

Keywords: Attachment Site (attB), Integrase, Oct-4 Promoter, PhiC31 Phage, Stem Cell

Ps-11: Reprogramming of Parental Genomes in Embryonic Stem Cell – Fibroblast Heterokaryons and Synkaryons

Gridina M.M*, Serov O.L

Developmental Biology, Novosibirsk, Russian Federation
Email: gridinam@yandex.ru

Objective: Up to date it was generally known that the hybrids, obtained after fusion of somatic cells with pluripotent cells, acquire various features of pluripotency from the embryonic stem (ES) cell fusion partner, including prolonged self-renewal ability, expression of pluripotency-specific genes, lack of expression of tissue-specific genes, developmental potential to contribute to the development of all three germ layers of the soma as well as to the germ cell lineage, and an undifferentiated epigenetic cell state. Heterokaryon formation (fused cells with two nuclei and a common cytoplasm) offers a unique tool to study the reprogramming process.
Materials and Methods: We developed design of experiment allowed us to analyze heterokaryons and synkaryons (hybrid cells) in a few hour's time after cell fusion. We fused the ES cells marked by GFP and fibroblasts labeled with fluorescent microspheres. Heterokaryons or hybrid cells were considered if they had both markers. Its reliability was confirmed using sequencing by presence of mitochondrial DNA derived from both parental cells.
Results: At 4-8 hours after fusion most heterokaryons had the fibroblasts-like morphology and were negative for alkaline phosphatase. The first hybrid cells appeared at 20 hours after fusion and were presented by both ES-like and fibroblasts-like cells which were prevalent. After a time about half of hybrid cells had ES-like morphology and formed colonies. In contrast to this, the fibroblasts-like hybrid cells didn't show self-renewal ability: they were unable to form colonies and grew as disconnected single cells. Immunofluorescent analysis demonstrated that all the hybrid cells with ES-like morphology were positive for expression of "pluripotent" genes: Oct4 and Nanog; negative for expression of genes typical for differentiated cells: lamin A/C, I-type Collagen and Fibronectin and contained active all parental X-chromosomes. However, in population of hybrid cells with fibroblast-like morphology and positive for fibroblast-specific genes (I-type Collagen and Fibronectin) there were a lot of hybrid cells which had intermediate phenotype: they did not express of Oct4, Nanog as well as I-type Collagen and Fibronectin and all X-chromosomes were active.
Conclusion: It is suggested that some cells underwent only partial reprogramming. Thus analysis of early events, which occurred directly after the fusion, showed that reprogramming process was more complex and it could not be described by "all or nothing" model.

Keywords: Heterokaryons, Cell Fusion, Reprogramming

Ps-12: Isolation and Dfferentiation of Mouse adult Pancreatic Ductal Stem Cells into Insulin-producing Cells In Vitro

Hatami L[1]*, Sumi S[2]

1. Biology Department, Zabol University, Zabol, Iran
2. Organ Reconstruction, Kyoto University, Kyoto, Japan

Email: hatlail@yahoo.co.uk

Objective: To isolate, culture and identify the mouse adult pancreatic ductal stem cells in vitro and to observe the potency of these multipotentional cells differentiation into insulin-producing cells.
Materials and Methods: Under anesthesia, mid line laparotomy incision was done then the proximal common bile duct is incised and cannulated with a catheter and injected with enzyme solution. After total pancratectomy, the pancreas is incubated in the enzyme solution. After isolation of pancreatic ductal stem cells followed culture in serum and serum free medium with additional Keratinocyte growth factor. The cells were induced by glucose. Then the cell types of undifferentiated and differentiated cells were identified using immunocytochemical and RT-PCR staining.
Results: The pancreatic ductal stem cells cultured in serum free medium grew very slowly. The identification of these cells was then identified by using the pancreatic ductal stem cell marker cytokeratin 19. But in the serum containing media, fibroblast very fastly grown and covered on pancreatic ductal stem cells. The immunoreactive staining and RT-PCR technique showed that pancreatic stem cells stimulated effectively to produce insulin producing cells in vitro.
Conclusion: This study revealed the expansion of adult mouse pancreatic ductal tissue in vitro. These cells can be cultured and induced by glucose and differentiated into insulin producing cells.

Keywords: Pancreatic Ductal Stem Cells, Differentiation, Insulin-Producing Cells, Mouse

Ps-13: Effect of CXCR1 and CXCR2 Receptors Inhibition on Megakaryocyting Differentitation of Umbilical Cord Blood CD 133+ Cells

Khalaf Adeli E[1]*, Kheirandish M[1], Abolghasemi H[1], Ebtekar M[2], Siadat S.D[3], Noroozi Aghideh A[4], Haghbin S[5], Dehghani B[1]

1. Research Center, Iranian Blood Transfusion Organization, Tehran, Iran
2. Immonology Department, Tarbiat Modares University, Tehran, Iran
3. Hepatitis and AIDS Department, Pasteur institute, Tehran, Iran
4. Laboratory Science, Army University of Medical Sciences, Tehran, Iran
5. Paediatrics Office, Shiraz University of Medical Sciences, Shiraz, Iran
Email: m.kheirandish@ibto.ir

Objective: Most studies have reported that some CXC chemokines including NAP-2, IL-8 and PF4 which expressed by megakaryocytes in megakaryocytopoiesis and affect cell expansion and differentiation of hematopoietic stem cells. Previous studies have shown that these chemokines inhibit megakaryocytopoiesis .The receptors for NAP-2 and IL8 and most likely PF4 were reported to be CXCR1 and CXCR2. The aim of this study was to investigate the effect of inhibition of CXCR1 and CXCR2 on differentiation of umbilical cord blood (UCB) CD133+cells into megakaryocyte progenitor cells.
Materials and Methods: Umbilical cord blood CD133+ cells were separated by magnetic cell sorting method. CD 133+ cells were placed immediately after selection, in a serum free medium supplemented with IL3, IL 6, TPO and stem cell factor (SCF) as well as 5% CO_2 for 12 days as a control cells. To investigate the effect of receptor inhibition, the CD133+ cells were cultured under a same condition with cocktail cytokine and divided into three groups: in the first group only CXCR1 was blocked , in the second group only CXCR2 blocked by neutralizing monoclonal antibody, in the third group both receptors were blocked by Anti CXCR1/CXCR2 neutralizing monoclonal antibodies. Expression of the CD 41 and CD61 antigen as megakaryocyte progenitor cell markers were evaluated by flow cytometry.
Results: The results showed that inhibition of CXCR1 and CXCR2 receptors together caused an increase in CD61 expression on days 7 and 12 in comparison to cells treated with IL3, IL 6, TPO and SCF ($p<0.05$). Inhibition of both receptors showed an increase in CD41 expression on days 7 and 12, but this increase was significant only on day 12 ($p<0.05$). Although inhibition of CXCR1 and CXCR2 alone augmented CD41 and CD61 expression on days 7 and 12, this increase was not significant ($p>0.05$).
Conclusion: CXCR1 and CXCR2 receptors play a potent role in the suppression of megakaryocytopoiesis through their ligands. We demonstrated that the inhibition of this suppressive effect can increase differentiation of UCB CD133+ cells into megakayocyte progenitor cells.

Keywords: CXCR1, CXCR2, Megakaryocytopoiesis, Umbilical Cord Blood (UCB) CD 133+ cell

Ps-14: Dedifferentiation of Granulosa Cells into Induced Pluripotential Stem Cells by Exposure to the Embryonic Stem Cell Extract

Kharazi E[1]*, Rohani L[1], Talaei T[1], Vojdani Z[1], Mostafavipour Z[1], Tabeie Z[2]

1. Anatomy, Shiraz University of Medical Sciences, Shiraz, Iran
2. Transplantation Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
Email: kharazie_ebrahim@yahoo.com

Objective: Epigenetic reprogramming of terminally differentiated cell can modify somatic cells to a pluripotential state. There are several approaches that induce pluripotency in somatic cells. Exposure the cells

with the embryonic stem cell extract is an easy way, and some investigations were done on fibroblast cell line. However, its efficiency was low. So, the objective of this study was to increase the number of reprogrammed granulosa cell as a full differentiated cell into pluripotential state.
Materials and Methods: human granulosa cells were cultured in medium containing azacytidine and trichostatine. The cells were exposed to mouse embryonic stem cells extract. The granulosa cells cocultured with mouse embryonic fibroblast in the presence of leukemia inhibitory factor. to assay the reprogrammed cells, alkaline phosphatase test and also immonocytochemistery were performed for OCT4, SOX2 and Nanog antibodies after 72 hours.
Results: The results indicated that granulosa cells showed the alkaline phosphatase activity. They also express OCT4, SOX2 and nanog after exposure to the embryonic stem cells extract.
Conclusion: It seems that the extract could induce dedifferentiation in granulosa cells. The previous research that was done on fibroblast cell line revealed the extract could lead the cells to express OCT4 but not nanog. We found that granolusa cells, as full differentiated somatic cells also can express the stem cell markers. It seems that the inhibitors of the methyl transferase and histone deacetylase could wipe the epigenetic markers and prepare the cells for reprogramming by administration of the extract.

Keywords: Reprogramming, Granulose Cell, Dedifferentiation, Azacytidin, Trichostatin, Embryonic Stem Cell Extract

Ps-15: Characterization and Genetic Manipulation of Human Umbilical Cord Vein Mesenchymal Stem Cells: Potential Application in Cell-Based Gene Therapy

Kermani A.J[1]*, Mowla S.J[1], Fathi F[2]

1. Department of Genetics, Faculty of Basic Sciences, Tarbiat Modares University, Tehran, Iran
2. KDRC, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran
Email: farfath@yahoo.com

Objective: Stem cells are defined by two main characteristics: self-renewal capacity and commitment to multi-lineage differentiation. The cells have a great therapeutic potential in repopulating damaged tissues as well as being genetically manipulated and used in cell-based gene therapy. Umbilical cord vein is a readily available and inexpensive source of stem cells that are capable of generating various cell types. Despite the recent isolation of human umbilical cord vein mesenchymal stem cells (UVMSC), the self-renewal capacity and the potential clinical application of the cells are not well known.
Materials and Methods: In the present study, we have successfully isolated and cultured human UVMSCs.
Results: Our data further revealed that the isolated cells express the self-renewal genes Oct-4, Nanog, ZFX, Bmi-1, and Nucleostemin; but not Zic-3, Hoxb-4, TCL- 1, Tbx-3 and Esrrb. In addition, our immunocytochemistry results revealed the expression of SSEA-4, but not SSEA-3, TRA-1–60, and TRA-1–81 embryonic stem cell surface markers in the cells. Also, we were able to transfect the cells with a reporter, enhanced green fluorescent protein (EGFP), and a therapeutic human brain-derived neurotrophic factor (hBDNF) gene by means of electroporation and obtained a stable cell line, which could constantly express both transgene
Conclusion: The latter data provide further evidence on the usefulness of umbilical cord vein mesenchymal stem cells as a readily available source of stem cells, which could be genetically manipulated and used in cell-based gene therapy applications.

Keywords: UVMSC, Cell Differentiation, Transfection, Self Renewal Genes, BDNF, GFP

Ps-16: Characterization of Chondrocyte Behaviors Affecting Quality of Cultured Cartilage

Khoshfetrat A.B[1]*, Kino-Oka M[2], Taya M[2]

1. Chemical Eng, Sahand University of Technology, Tabriz, Iran
2. Biotechnology, Osaka University, Osaka, Japan
3. Chemical Eng, Osaka University, Osaka, Japan
Email: khoshfetrat@sut.ac.ir

Objective: Chondrocyte cells exhibited different behaviors when cultured at low seeding density (X0=2.0×105 cells/ cm^3), as compared to high seeding density (X0=2.0×106 cells/ cm^3) in collagen gels to generate tissue-engineered cartilage (1). The cells in the low-density culture migrated to form loose aggregates, showing less production of collagen type II. In this research, we examined morphological behaviors of chondrocyte cells in more detail and evaluated quantitatively the effect of seeding density on the migration of chondrocytes in collagen gels. Since communications among chondrocytes via autocrine/paracrine signaling can affect behaviors of the cells, we also evaluated the effect of a well-known chondrogenic growth factor, transforming growth factor-beta1 (TGF-beta1), on the migration of chondrocytes in collagen gels and architecture of cultured cartilage.
Materials and Methods: Rabbit chondrocyte cells were cultured at different seeding densities, ranging from X0=1.0×105 to X0=1.6×106 cells/cm3, in collagen gels. Cytoplasm and collagen type II were stained, and stereoscopic observation was performed by using confocal laser scanning microscopy (CLSM) to define the migrating cells by sphericity values (Sc) of individual cells at 5 days. The cultured cartilages were observed

at 10 days after seeding using CLSM in terms of spatial distribution, morphology and collagen type II formation. To study the effect of TGF-beta1 on chondrocyte behaviors and architecture of cell aggregates, the cells were also cultured at low seeding density (X0=2.0×105 cells/cm3) for a 14-day period and exposed to various TGFβ1 concentrations in range of 0 to 10 ng/ml. The quantitative evaluation of morphology and the histological observation of cultured gel were carried out at culture days of 5 and 14, respectively. Gene expression analysis of migration- and differentiation-related genes was also performed by real time RT-PCR to support the morphological evaluation of the cell behaviors in the research.

Results: The chondrocytes underwent a transition to a spindle-shaped morphology, then started to transform again to original phenotype after migration and gathering in the starburst aggregates which was accompanied by poor production of collagen type II in the culture seeded at X0=1.0×105 cells/cm3 during a 10-day culture period. In contrast, the cells proliferated normally in the dense aggregates of semilunar-shaped cells with rich excretion of collagen type II in the culture seeded at X0=1.6×106 cells/cm3. Quantitative evaluation of morphology at 5 days also revealed that the frequency of migrating cells (Sc<0.95) in the culture seeded at X0=1.0×105 cells/cm3 was 0.25, the value of which was 25 times higher than that at X0=1.6×106 cells/cm3. These results suggest that seeding density is a factor to cause variation of the quality of cultured cartilage by modulation of the migration and aggregation of chondrocytes in the collagen gels (2). The frequency of migrating cells with Sc<0.95 increased in dose-response to TGF-beta1. The histological observation of cultured gels at 14 d also revealed that the starburst aggregates with the spindle-shaped cells emerged in the TGF-beta1-free culture, accompanying the poor production of collagen type II, whereas the spherical-shaped cells were observed in the starburst aggregates with rich excretion of collagen type II in the culture with 5.0 ng/ml TGF-beta1. These results suggest that TGF-beta1 has a culture-phase dependent influence on the morphological characteristics of the chondrocytes cultured in collagen gels (3). Gene expression analysis coincided with the results obtained from the quantitative evaluation of morphology and the observation of cultured cartilage.

Conclusion: We demonstrated that the behaviors of cell division, migration, gathering and differentiation caused spatial heterogeneity in the fate of cell aggregates in terms of distribution, size, morphology as well as collagen type II formation. The present research reveals the importance of characterization of cell behaviors coordinated from cell communications to regulate the architecture of cell aggregates, and subsequently govern the quality of cultured cartilage.

Keywords: Chondrocyte Cells, Low Seeding Density, Migration, Aggregate Formation, TGF-beta1

Ps-17: Proliferation and Aging of Rat Mesenchymal Stem Cells from Epicardial Adipose Tissue in Comparison to those from Bone Marrow Tissue

Mardpour S*, Baghaban Eslaminejad M.R, Ebrahimi M

Department of Stem Cells and Development Biology, Royan Institute for Stem Cells Biology and Technology, ACECR, Tehran, Iran
Email: souri_mardpour@hotmail.com

Objective: Mesenchymal stem cells (MSCs) have first been isolated and described from bone marrow tissue. Subsequent studies have indicated the existence of these cells in many other tissues. Little is known regarding the differences between MSCs from different sources. In this study MSCs from adipose tissue (AT) were compared to those from bone marrow (BM) samples.

Materials and Methods: For this purpose AT was obtained from rat epicardial tissue and subjected to enzymatic digestion. Released cells were then plated and culture expanded through several passages. In parallel, expansion cultures were established for rat BM cells for several subcultures. To ensure the MSCs nature, isolated cells were differentiated among bone, cartilage and adipose cell lineages. To compare the proliferation rate of the cells from two sources, population doubling time (PDT) and colonogenic activity were determined for either culture. Beta galactosidase assay was used to compare the MSCs from two sources in terms of their culture senescence.

Results: Culture-expanded cells from either source were able to produce bone, cartilage and adipose cell lineages (evidenced by RT-PCR analysis for related specific genes) confirming that they were the MSCs described elsewhere. According to our results AT cells appeared to have a short PDT value than their BM counterparts (45.84 ± 16 versus 318.24 ± 18.24, p<0.05) suggesting that they were more proliferative compared to BM-MSCs. Also, AT cells tended to form statistically large colonies and more colony numbers than their BM counterparts (p<0.05). The more important point was that AT cells contained significantly less beta-galactosidase positive cells compared to those in BM cell cultures (22 ± 2.9 versus 54 ± 2.27, p<0.05).

Conclusion: Taken together, these data suggest that MSCs derived from AT are appropriate cell source for stem cell applications, because they were apparently more proliferative and their cultures possess more less cells undergoing senescence changes compared to MSCs from bone marrow tissue.

Keywords: Epicardial Adipose Tissue, Bone Marrow, Proliferation, Senescence

Ps-18: Intravenous Injection of Human Umbilical Cord Matrix stem Cell (Wharton Jelly Stem Cell) Provides Functional Recovery in a Rat Model of Traumatic Brain injury

Marzban M*, Bakhtiary M, Joghataei M.T, Mehdizade M, Modiry N

Anatomy, Iran University of Medical Science, Tehran, Iran
Email: mohsen136051@yahoo.com

Objective: This study was designed to examine the effects of human umbilical cord matrix stemcell (hUC-MSC) administration in rats for 6 week after traumatic brain injury (TBI).
Materials and Methods: Adult male Wistar rats (n = 30) were injured with controlled cortical impact device and divided into Three groups. The treatment groups (n = 10 each) were injected with 2 × 106 (2 milion) intravenously, and whicle group (n = 10) received phosphate buffered saline (PBS). whereas the control group (n = 10) receive nothing All injections were performed 1 day after injury into the tail veins of rats. All cells label with Brdu before injection into the tail veins of rats. Neurological functional evaluation of animals was performed before and after injury using Neurological Severity Scores (NSS). Animals were sacrificed 6 week after TBI and brain sections were stained by Brdu immunohistochemistry.
Results: Statistically significant improvement in functional outcome was observed in treatment groups when compared with control ($p < 0.01$). This benefit was visible 1 week after TBI and persisted until 6 week (end of trial).Histological analysis showed that (hUCMSC) were present in the lesion boundary zone at 6 week with all cell injected animal
Conclusion: hUCMSC injected in rats after TBI survive until 6 week and provide functional benefit.

Keywords: Stem Cell, Brain Injury, Wharton Jelly

Ps-19: Evaluation of Heat Shock Protein 90 and Heat Shock Constitutive Protein7o Expression in Cultured Limbal Stem Cells During Air Exposure

Mohamadi P[1]*, Ebrahimi M[1], Baharvand H[1, 2]

1. Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran
Email: mohamadi@royaninstitute.org

Objective: Heat shock proteins (Hsps) are highly conserved proteins which have important roles in protection against stress, signaling pathway and differentiation, embryonic development and chaperon activity. The aim of this study is investigation of HSP90 and HSC70 expression before and after air exposure in cultivated limbal stem cells on denuded amniotic membrane(AM) and plastic dish(PD).
Materials and Methods: Limbal biopsies were taken from normal human limbus (Central Eye Bank of Iran), and were cultured as an explant on amniotic membrane and plastic dish. Cells were exposed to the air after 14 days of culture. The expression of proposed limbal stem cells markers (p63,ABCG2),corneal markers(K3/12,Connexin43) and also HSPs(90,hsc70) were analyzed by RT-PCR, immunocytochemistry and flowcytometry pre and post air exposure at day 14 (before air exposure) and 30(after air exposure) respectively.
Results: ABCG2 positive cells with high expression of p63 and low expression of K3/K12 and Connexin43 were considered as limbal stem cells while ABCG2 negative cells with low expression of p63 and high expression of K3/K12,Connexin43 were assumed as corneal cells. Our data showed that HSPs expressed strongly at mRNA level before and after air exposure while they expressed in different pattern at protein level. It was interested that HSP90 proteins were expressed weakly in limbal stem cells at day 14 on both groups, but its expression increased after air exposure at day 30($p<0.05$) only in AM group . on the other hand HSC70 were expressed strongly on plastic dish in compared with amniotic membrane ($p<0.05$) before air exposure but after air exposure its expression has significant increasing in AM group.
Conclusion: We assumed hsp90 has important role during differentiation process but only in early stage of differentiation because its increasing was in AM group,and in this group we had TAC cells(early differentiated cells) after air exposure but Hsc70 expression may related with differentiation process in both early and terminally stage of differentiation in cultured limbal stem cell.

Keywords: Limbal Stem Cell, Amniotic Membrane, Air Exposure, Heat Shock Protein, Plastic Dish

Ps-20: Gene Expression of Catalase during Neural Differentiation of P19 Cells

Mojbafan M[1, 2]*, Ghaedi K[1, 2], Razavi S[3], Tanhaei S[1], Karamali F[1], Rabiee F[1], Nasr Esfahani M.H[1]

1. Department of Cell and Molecular Biology, Royan Institute for Animal Biotechnology, ACECR, Isfahan, Iran
2. Department of Molecular Biotechnology, Royan Institute for Animal Biotechnology, ACECR, Isfahan, Iran
3. Anatomy, Isfahan University of Medical Sciences, Isfahan, Iran
Email: marziyeh_mojbafan@yahoo.com

Objective: The P19 murine embryonal carcinoma (EC) cell line is a valuable in vitro model cell that can be differentiated into neurons by cellular aggregation in presence of the differentiating agent retinoic acid (RA).
Materials and Methods: Total RNA from P19 cells were extracted and cDNA was synthesized. Using specific primers, respective cDNAs were amplified and were analyzed by semi-quantitative RT-PCR

Results: In this project, a peroxisomal gene such as catalase has been selected and the profile of its expression has been investigated in P19 cells. Expression of peroxisomal gene like Catalase, as peroxisomal matrix protein in comparison with pluripotency markers such as Oct4 and Nanog, neural markers such as Pax6, Ngn-1, Map2 and a house keeping gene such as β-tubulin have been investigated by RT-PCR
Conclusion: Data indicated that during neural differentiation, expression of pluripotency markers have been down regulated while, expression of neural markers was significantly increased. However incase of Catalase gene expression, there was an increase in Catalase gene expression upon Retinoic acid treatment, during neural differentiation, which was observed at the final steps of neurogenesis.

Keywords: Catalase, Neurogenesis, Oct4, P19

Ps-21: *In Vitro* Differentiation of Human Bone Marrow-Derived Mesenchymal Stem Cells into Cardiomyocytes

Motamedy R[1]*, Azadbakht M[1], Fathi F[2], Amini A[1], Raoufi A[1], Amini S[2]

1. Faculty of Science, RAZI University, Kermanshah, Iran
2. Kurdistan University of Medical Sciences, Sanandaj, Iran
Email: rmotamedy@gmail.com

Objective: The inability of adult cardiomyocyte to divide to a significant extent an regenerate the myocardium after injury leads to permanent deficits in the number of functional cells, which can contribute to development and progression of heart failure. Transplantation of stem cells into the injured myocardium is a novel and promising approached in the treatment of cardiac disease and the restoration of myocardial function. In the present study we investigated the potential of human mesenchymal stem cells from adult bone marrow to differentiate into cardiomyocytes.
Materials and Methods: . Human Bone Marrow Mesenchymal Stem Cells (hBMSCs) cultured in enriched medium. hBMSC were treated with 10-6M oxytocin for one month. Morphologic characteristics were analyzed by phase contrast microscope. Expression of hα3-actinin and hβMHC (myosin heavy chain beta) and OTR (Oxytocin Receptor) was detected by RT-PCR. Protein expression of α-actinin and Troponin¬I-C was analyzed through immunostaining. hBMSCs were spindle-shaped with irregular processes.
Results: Cell treated with oxytocin were connect with adjoining cells forming myotube like structures. Immonostaining of the differentiated cells for α-actinin and Troponin¬I-C were positive.
Conclusion: Based on these observations, we conclude that hBMSCs retain cardiomyogenic potential suitable for cell therapy against intractable heart diseases.

Keywords: Human Bone Marrow Mesenchymal Stem Cells, Cardiomyocyte, Cell Differentiation

Ps-22: Experimental Demyelination in Adult Rat Optic Chiasm and Nerve Mobilizes Endogenous Neural Stem Cells from the Lateral Subventricular Zone and Rostral Migratory Stream

Mozafari S[1]*, Sherafat M.A[1], Javan M[1], Mirnajafi-Zadeh J[1], Tiraihi T[2]

1. Medical Physiology, Tarbiat Modares University, Tehran, Iran
2. Anatomy Department, Tarbiat Modares University, Tehran, Iran
Email: az.raoufi@gmail.com

Objective: Adult mammalian brain contains multipotent neural stem cells reside mainly in germinal zones including subventricular zone (SVZ) of lateral ventricle and rostral migratory stream (RMS). Identifying cells with the capacity to generate oligodendrocytes in the adult CNS and assessment of their migrating potential would help in the development of strategies to promote endogenous remyelination. Regarding to vulnerability of optic apparatus, particularly optic chiasm and nerves in Multiple Sclerosis, here, it was tried to determine whether endogenous neural stem cells from these germinal zones are able to mobilize in response to the experimental local demyelination in adult rat optic chiasm and nerves?
Materials and Methods: We profited from an improved demyelination model which is able to built-in rat optic chiasm and nerves simultaneously following stereotaxic microinjection of lysolecithin (LPC) into the chiasm without undesirable distribution. Histological and functional verifications of the model and the repair assessment were accomplished using special myelin staining and visual evoked potential recording. Gene expression level for MBP, Olig2 and GFAP; consequently as mature oligodendrocytes activity, reactive oligodendrocyte precursor cells and astrocytes activity, were assessed at the site of lesion at 2, 7, 14 and 28 days post induction (dpi). Endogenous adult stem cell tracing was performed using intraperitoneal administration of BrdU prior to the gliotoxin injection and assessment of antigenisity against Nestin, immunohistochemicaly.
Results: Demyelination was considerable in days 7 and 14 and an incomplete remyelination occurred in 28 dpi. MBP gene expression was decreased significantly on day 7 post induction, but Olig2 and GFAP gene expression were increased at this time-point. These changes, then, were slowly reversed at days 14 and 28. Because in control animals BrdU labeled cells were restricted to the SVZ and RMS, their presence in structures other than the SVZ and RMS implies that they originate from the SVZ or RMS. Two days post induction, number of BrdU+ cells in SVZ and RMS were increased and 7 dpi, these cells were left SVZ and RMS and located

in the brain parenchyma with a cell gradient in which more number of cells placed near the SVZ and RMS and distributed toward the demyelinated area. Nestin, a neural stem cell marker was up-regulated at the site of injury and also in walls of lateral ventricle at 2 dpi and reached to a maximum level at 7 dpi which then was sustained in tissue with lower expression by time. Double staining studies on lateral ventricle walls on day 7 post induction was revealed that only a few Nestin+ cells were also BrdU+.
Conclusion: SVZ and RMS endogenous adult neural stem cells are able to be recruited by existence of experimental demyelination even in the adult white matters like optic chiasm and nerves where locate far from the lateral ventricles and are commonly affected by MS.

Keywords: Remyelination, Endogenous Adult Neural Stem Cells, SVZ, RMS, Cell Migration, Rat

Ps-23: A Protocol for Isolation and Culture of Mesenchymal Stem Cells from Mouse Bone Marrow

Samad Nadri[1]*, Masoud Soleimani[2]

1. Shahid Beheshti of Medical Science University, Tehran, Iran
2. Hematology, Tarbiat Modares University, Tehran, Iran
Email: nadrisamad@yahoo.co.uk

Objective: We explain a protocol for straightforward isolation and culture of mesenchymal stem cells (MSCs) from mouse bone marrow to supply researchers with a method that can be applied in cell biology and tissue engineering with minimal requirements. Our protocol is mainly based on the frequent medium change in primary culture and diminishing the trypsinization time.
Materials and Methods: Mouse mesenchymal stem cells (mMSCs) are generally isolated from an aspirate of bone marrow harvested from the tibia and femoral marrow compartments, then cultured in a medium with DMEM and FBS, for 3 hours in a 37°C-5% CO_2 incubator. Non-adherent cells are carefully removed after 3 hours and fresh medium is replaced. When primary cultures become nearly confluent, the culture is treated with 0.5ml of 0.25% Trypsin containing 0.02% EDTA for 2 minutes at room temperature. For confirmation mesenchymal nature, the cells were induced to differentiate along osteoblastic, adipogenic, chondrogenic. Furthermore, the expression of some surface antigens was investigated.
Results: Cells isolated using this method demonstrated the MSCs characteristics including their ability to differentiate into mesenchymal lineages. The cells retained the differentiation potentials in expanded cultures up to 10 passages. The cells were reactive to the CD44, Sca-1, and CD90 cell surface markers. The cells were negative for the hematopoietic surface markers such as CD34, CD11b, CD45, CD31, CD106, CD117 and CD135.
Conclusion: A purified population of MSCs can be obtained three weeks after the initiation of culture.

Keywords: Mouse Mesenchymal Stem Cells, Bone Marrow, Surface Antigens

Ps-24: Proteomic Analysis of Monkey Embryonic Stem Cell during Differentiation

Davood Nasrabadi[1]*, Mehran Rezaei Larijan[1], Leila Pirhaji[1], Hamid Gourabi[1, 2], Abdolhossein Shahverdi[1], Hossein Baharvand[1, 2, 3], Ghasem Hosseini Salekdeh[1, 2, 4]

1. Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Genetics, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran
4. Department of Physiology and Proteomics, Agricultural Biotechnology Research Institute of Iran, Karaj, Iran
Email: davood_bn@yahoo.com

Objective: Identification proteins involved in stem cell differentiation.
Materials and Methods: We applied a 2-DE based proteomic approach coupled with mass spectrometry to identify genes controlling monkey ESCs proliferation and differentiation. We analyzed proteome of ESCs during proliferation and different stages of spontaneous differentiation (day 3, 6, 12, and 30) by embryoid body formation.
Results: Out of about 663 (15 protein spots reproducible detected on gels, 127 proteins showed significant changes during differentiation. Mass spectrometry analysis of differentially expressed proteins resulted in identification of 95 proteins involved in cell cycle progression and proliferation, cell growth, transcription and chromatin remodeling, translation, metabolism, energy production and Ras signaling. In addition, we created protein interaction maps and distinctly different topology was observed in the protein interaction maps of the monkey ESC proteome clusters compared with maps created using randomly generated sets of proteins
Conclusion: Taken together, the results presented here revealed novel key proteins and pathways that are active during ESC differentiation.

Keywords: Proteomics, Embryonic Stem Cells, Differentiation, Monkey, Interaction Network

Ps-25: Differentiation of Rat Bone Marrow Mesenchymal Stem Cell into Hepatocyte-Like Cells in Natural Scaffolds and Transplantation into Liver-damage Rat

Nasiri Z[1]*, Soleimani M[2], Pedram M[3], Sasani F[4], Mosavi Z[4]

1. Animal Physiology, Tehran University, Tehran, Iran
2. Hematology, Tarbiat Modares University, Tehran, Iran
3. Clinical Science, Veterinary Medicine, Tehran, Iran
4. Pathology, Veterinary Medicine, Tehran, Iran
Email: zahran29@gmail.com

Objective: Tissue engineering is a promising approach to developing hepatic tissue suitable for the functional replacement of a failing liver .previous study have been showed that Bone marrow mesenchymal stem cells (BMSc) have plasticity to differentiate into hepatocyte-like in monolayer . The aim of the present study was to investigate in vitro study was to evaluate the morphology and hepatocyte differentiation of bone marrow derived mesenchymal stem cells with in natural scaffold that could influence the proliferation rate and survival of rat hepatocytes both during long term culture and after in vivo transplantation.
Materials and Methods: In the present study, BMSCs was isolated from rat male SD (3-4 weeks) Marrow-derived hepatocyte were seeded into porous natural coral scaffolds in a density of 1 × 106/mL in 300 µL cell suspension, cell cultured were treated with HGF, EGF, Dexamethasone and OSM in two step protocol and an animal model of engraftment in Fibrosis liver induced using carbon tetrachloride (CCl4) in rats .after 14 day's tissue-engineered hepatocyte valves were analyzed by RT-PCR and scanning electron microscopy Proliferation of the seeded cells on the scaffolds was detected using the MTT assay. After 8-10 wk of MSCs administration, blood samples were collected to measure the albumin, SGOT, SGPT concentration all rats were killed and fibrosis index were assessed by histopathology.
Results: SEM micrographs of cells showed that cells adhered and proliferated well on the outer and inner surfaces of these natural scaffolds the result of immunofluorescent analysis revealed the expression of albumin (ALB) and alpha feto protein (AFP) and anti –hepatocyte from day 7 to day 28 similar the finding of expression of Alb ,CK19 AFP,…mRNA by RT-PCR. In vivo results demonstrated the biocompatibility of natural scaffold implanted in rat fibrosis and significantly increased the serum albumin concentration and vascularization of porous scaffolds implanted on liver lobes and improved hepatocyte engraftment.
Conclusion: All these results indicate that rMSCs differentiated into hepatocyte in natural scaffold, Implantable engineered liver tissue using natural coral as scaffold for growth and differentiation of hepatocytes has been developed. This technique is an attractive tool for the development of liver tissue engineering and may provide a source of differentiated cells for treatment of liver diseases.

Keywords: Hepatocyte, Differentiation, Natural Scaffold, Msenchymal Stem Cell

Ps-26: Production and Maintenance of Two Populations of Neural Progenitor Cells with Rostral and Caudal Properties from Human Embryonic Stem Cells

Nemati Sh[1]*, Hatami M[1], Hemmesi K[1], Kiani S[1], Baharvand H[1, 2]

1. Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran
Email: shivanemati2004@yahoo.com

Objective: Availability of human embryonic stem cells (hESC) has enhanced human neural differentiation research. While differentiation is directed towards the neural lineage, lack of an optimal protocol implies generation of other lineage cells as contaminants. Thus, one major challenge in the field is to generate a homogeneous and renewable, easy to culture, neural progenitor (NP) cell population committed to the neural lineage, capable of serving as an unlimited lineage-restricted cell source for replacement therapy and novel drug screening and/or for other studies.
Materials and Methods: Here, we present two pure populations of long-term self-renewing rosette-type hESC-derived neural progenitor cells (hES-NP cells) with Rostral and Caudal properties induced by retinoic acid and noggin(RA+ population & RA- population), which exhibit extensive self-renewal, clonogenicity, and stable neurogenesis
Results: Flowcytometry and immunocytochemistry analysis of both populations showed increase in expression of Nestin, SOX-1, and Pax-6.Then real-time PCR analysis showed increase expression of Hoxc5 and Otx2, in RA+ population rather than RA- population. In addition, hES-NP cells maintained their developmental potential through long-term storage in liquid nitrogen and multiple freeze–thaw cycles.
Conclusion: These results demonstrate that hES-NP cells have the ability to provide an expandable and unlimited human cell source that can develop into specific neuronal and glial subtypes.

Keywords: Human Embryonic Stem Cells, Neural Progenitor, Rosette-Type hESC-Derived Neural Progenitor Cells

Ps-27: Expression of the Stem Cell Marker CD133 in Breast Carcinoma

Nikbakht Dastjerdi M

Anatomical Sciences, Isfahan University, Isfahan, Iran
Email: nikbakht@med.mui.ac.ir

Objective: Although the primitive haematopoietic and neuronal stem cell marker CD133 is known to be present in cancer stem cells in breast carcinoma, the

prognostic impact of CD133 in breast cancer patients remains limited.
Materials and Methods: The expression of CD133 protein was analysed by immunohistochemistry in 96 breast cancer specimens and the association of CD133 expression with clinicopathological characteristics, tumour recurrence and survival of the patients was evaluated.
Results: Immunohistochemical analysis of 96 breast cancer tissue specimens revealed that CD133 positive tumour cells were frequently present in breast cancer. Increased CD133 immunostaining was found in 41.3%. Increased CD133 expression levels were correlated with increased tumour grade. Kaplan–Meier analysis indicated that patients with increased CD133 levels had shorter overall survival and higher recurrence rates compared with patients with low CD133 expression. Multivariate analyses revealed that increased CD133 expression was an independent prognostic factor for survival and tumour recurrence in patients with breast cancer.
Conclusion: These findings suggest that reactivated CD133 positive cells are frequently present in breast cancer. Additionally, increased CD133 expression corresponds with higher grade tumors in breast cancer , thus indicating a poor prognosis for patients.

Keywords: Stem Cell Marker, CD133, Breast Carcinoma

Ps-28: Cancer Stem Cell Marker is Enriched in APC Deficient Colorectal Tumors

Nikbakht Dastjerdi M*

Anatomical Sciences, Isfahan University, Isfahan, Iran
Email: nikbakht@med.mui.ac.ir

Objective: Colorectal cancer cells with a CD44+ phenotype have been suggested to have tumor-initiating properties with stem cell-like and invasive features, although it is unclear whether their presence within a tumor has clinical implications. We have explored the prevalence of cells with different CD44 phenotypes within colorectal cancer subtypes.
Materials and Methods: Immunohistochemistry was used to quantify CD44 expression in 140 human colorectal tumors for which information on other tumor markers was available.
Results: A considerable heterogeneity in CD44 expression was seen both between and within tumors. A complete lack of this protein was evident in 35% of the tumors. CD44+cells were detected in 65% of the tumors, ranging in proportion from only a few to close to 100% of tumor cells. The CD44+phenotype was most common in the basal-like subgroup – characterized as negative for ANXA1, and as positive for bcl2 and particularly common in APC defficient tumors, of which 94% contained CD44+cells.
Conclusion: We demonstrate an association between APC defficient colorectal cancer and the presence of CD44+cells. Not all basal-like tumors and very few ANXA1 tumors, however, contain CD44+cells, emphasizing that a putative tumorigenic ability may not be confined to cells of this phenotype

Keywords: Stem Cell Marker, CD44, APC, Colorectal Cancer

Ps-29: The Expression of Pluripotency Stem Cell Marker in Ovarian Cancer

Nikbakht Dastjerdi M*

Anatomical Sciences, Isfahan University, Isfahan, Iran
Email: nikbakht@med.mui.ac.ir

Objective: Oct 3/4 (Octamer 3/4), a member of POU family has been considered as an important stem cell marker and essential transcription factor during human embryogenesis. In recent years, there have also been reports on presence of Oct 3/4 in differentiated benign and malignant human cells. The objective of this study was to investigate the transcription and the protein expression of Oct 3/4 isoforms in ovarian cancer.
Materials and Methods: 33 ovarian cancer specimens studied. The transcription of Oct 3/4 was analyzed using RT-PCR approach associated with restriction digestion analysis. Oct 3/4 protein expression was studied by immunohistochemistry
Results: We detected expression of type 1 of Oct 3/4 as well as protein expression with nuclear localization of Oct 3/4 isoform 1. The stem cell markers CD44 also were detected in Oct 3/4 immunopositive cells
Conclusion: Our results indicate that only the nuclear isoform 1 of Oct 3/4 is present in ovarian cancer. The malignant cells, which are immunopositive for variant 1 of Oct 3/4, also expressed another stem cell marker CD44 supporting that variant 1 of Oct 3/4 is a pluripotency marker.

Keywords: Pluripotency, Oct 3/4, Ovarian Cancer, CD44

Ps-30: Platelet Growth Factors Suppress the Expansion, but Promote the Differentiation of Cord Blood CD133+ Stem Cells to Megakaryocyte Progenitors

Noroozi Aghideh A[1]*, Kheirandish M[1], Abolghasemi H[1], Gharabaghian A[1], Khalaf Adeli E[1], Siadat S.D[2], Haghbin S[3]

1. Research Center, Iranian Blood Transfusion Organization (IBTO), Tehran, Iran
2. Pasteur Institute, Tehran, Iran
3. Shiraz University of Medical Science, Shiraz, Iran
Email: Noroozi_1895@yahoo.com

Objective: Umbilical cord blood (UCB) is a rich source of hematopoietic cells as a valuable source for hematopoietic stem cell transplantation (HSCT). In addition, cord blood megakaryocytic progenitor cells are more immature than other sources such as bone marrow (BM) that result in delayed platelet recovery in the recipients of UCB transplants. Platelet rich plasma (PRP) is a concentrate of platelet growth factors and many studies have shown the effects of these factors on the expansion and function of different cell types. In this study, we surveyed the effects of platelet growth factors including the platelet supernatant (PS), platelet lysate (PL) and activated PRP (AP) on the expansion and differentiation of cord blood CD133+ stem cells into megakaryocytic progenitor cells.
Materials and Methods: UCB 133+ cells were separated by MACS method and counted using improved neubauer hemocytometer. their viability was determined using dye exclusion assay and 7-AAD method. AP was prepared by PRP activation by thrombin and calcium chloride. PS and PL preparation was performed by high speed centrifugation (900 g) of PRP and freezing and thawing of PRP, respectively. The growth factors and protein concentrations in these platelet products were measured by ELISA and Bradford methods, respectively. Mean expression rate of CD133, CD41, CD61 and CD42b in day 0 and CD41, CD61 and CD42b at the end of culture period were assayed by flow cytometry.
Results: The results showed that PDGF and TGF-β concentrations are higher than other platelet growth factors. Further, protein concentrations in PS, PL and AP were 460, 490 and 480 mg/ml, respectively ($p<0.05$). AP at 2,10,25,50 mg/ml and PS at 50 and 100 mg/ml concentrations significantly suppressed the expansion of CD133+ cells in the first day of the culture ($p <0.05$). This suppression for PL at 50mg/ml and for AP at 10, 25 and 50 mg/ml concentrations after the fourth day of culture was not significant ($p>0.05$). On the other hand, the expression of CD41, CD61 and CD42b markers in the presence of all growth factors increased compared with the control media, but only AP at the 10, 25, 50 mg/ml concentrations in the first day of culture showed a significant effect on the expression of CD41 and CD61 markers ($p<0.05$). However, increasing in the expression of CD42b as a late marker of megakaryocytic lineage was not significant under none of the above conditions.
Conclusion: Taken together, platelet growth factors suppressed the expansion of UCB CD133+ cells and increased the differentiation of UCB CD133+ cells into megakaryocytic progenitor cells in a dose and time dependent manner. In our study, amplifying of UCB CD133+ cells by cytokine cocktail and then promotion of their differentiation using platelet growth factors appeared to be an efficient strategy to overcome the low numbers in UCB units that limits their use in full reconstitution of adult BM.

Keywords: Umbilical Cord Blood, CD133+ Cells, Megakaryocyte Progenitors, Expansion, Differentiation, Platelet Growth Factors

Ps-31: Effects of Dexamethasone (Dex) on Apoptosis in the Mouse Testicular Germ Cells

Orazizadeh M*, Hashemitabar M, Khorsandi L.S

Department of Anatomical Sciences, Ahvaz Jundishapoor University of Medical Sciences, Ahvaz, Iran
Email: M_Orazizadeh@yahoo.com

Objective: The aim of the present study was to find out whether Dexamethasone (Dex), a widely used GC, would influence the apoptosis and expression of Bax, an important proapoptotic protein, in the mouse testicular germ cells.
Materials and Methods: Experimental groups of 8 male NMRI mice received one of the following treatments daily for 7 days: 4, 7 and 10 mg/kg Dex. Control groups were treated with equivalent volumes of saline. Experimental and control animals were sacrificed 24 h after the last injection. Immunohistochemical procedure was applied to evaluation of Bax expression and statistically evaluated. The deoxyuridine nick-end labeling (TUNEL) was carried out to assessment of the apoptotic germ cells.
Results: Apoptotic index was significantly increased in 7 and 10 mg/kg Dex treated mice ($p<0.05$). Bax expression was upregulated mainly at stages VII-VIII of spermatogenic cycle in experimental groups ($p<0.05$).
Conclusion: It appears that GCs such as Dex could induce apoptosis through the expression of proapoptotic proteins.

Keywords: Apoptosis, Dexamethasone, Germ Cells, Bax, Spermatogenesis

Ps-32: Induction of PEP Gene Expression by Retinoic Acid during Mouse Embryonic Stem Cell Neurogenesis

Ostadsharif M[1]*, Ghaedi K[2, 3], Nasr Esfahani M.H[2], Tanhaei S[2], Karbalaii K[2]

1. Basic Medical Sciences Department, School of Nursery and Midwifery, Islamic Azad University, Khorasgan Branch, Isfahan, Iran
2. Department of Cell and Molecular Biology, Royan Institute for Animal Biotechnology, ACECR, Isfahan, Iran
3. Biology Department, School of Sciences, University of Isfahan, Isfahan, Iran
Email: maryam.ostadsharif@gmail.com

Objective: Retinoic acid (RA), the natural acidic derivative of vitamin A, can modulate the expression of specific genes and can induce some cell types, such as the mouse embryonic stem cells (mESCs), to differentiate in culture. mESCs treated with retinoic acid are in-

duced to differentiate into neuron-like cells. One of the recently identified peroxisomal matrix proteins is peroxisomal protein (PeP). Previous studies have showed PeP in the adult mouse is mainly expressed in heart, skeletal muscle, and brain tissues. This expression pattern suggests a possible role for PeP in processes of neurogenesis. Therefore, the aim of this study was to assess the expression of PEP during neurogenesis.
Materials and Methods: We performed a semiquantitative RT-PCR analysis to study of PEP mRNA expression in RA-treated and non-RA-treated in mouse embryonic stem cells (mESCs). Therefore mESCs cells were cultured for process of neurogenesis. The process of cell differentiation was chased and verified by immunostaining and gene expression of marker proteins for different stages of neurogenesis.
Results: The data indicated that PEP mRNA expression level increased after 4 days treatment by RA significantly, while there was no increment in the level of PeP mRNA in untreated cells. Moreover, PeP gene expression was decreased in neural cells.
Conclusion: This result indicated that the expression of PEP gene is inducible by retinoic acid treatment during the stage of neurogenesis. Thus further studies should be done to demonstrate the main function of PeP gene in this stage.

Keywords: Mouse Embryonic Stem Cells, Retinoic Acid, PEP Expression

Ps-33: Generation of Oligodendrocyte Precursors from Human Induced Pluripotent Stem Cells

Pouya A.R[1]*, Rezaei Larijani M[1], Taei A[1], Seifinejad A[1], Totonchi M[1, 2], Aghdami N[1], Baharvand H[1, 2]

1. Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran
Email: ar_pouya@yahoo.com

Objective: Induced pluripotent stem (iPS) cells have opened a new area for biological and clinical researches. The characteristics of iPS cells are remarkably similar to embryonic stem (ES) cells. However, detailed differentiation properties and the directional differentiation system of iPS cells have not been demonstrated into oligodendrocyte precursors. This study aimed to differentiate human iPS cells into oligodendrocyte precursors. into oligodendrocyte precursors.
Materials and Methods: The protocol consisted of an induction of neural-lineage cells by exposing cultures to retinoic acid simultaneous with the preferential selection of oligodendroglial-lineage cells by media components and epidermal growth factor, and the differentiation factor triiodothyroidin hormone (T3).
Results: Morphological features and molecular analysis including immunocytostaining consist of Olig2, A2B5, PDGFα-R, NG2, O4 and GalC plus other neural type cell's markers and RealTime-PCR for Oligodendrocyte markers showed that human iPS and hES cells generate oligodendrocyte precursors.
Conclusion: This study showed that oligodendrocyte precursors and their derivatives can be generated from human iPS cells. The ability to generate human oligodendroglia from iPS will provide a means to generate autologous donor cells for transplantation therapies once the safety issue is overcome.

Keywords: Human iPS, Differentiation, Oligodendrocyte Precursor

Ps-34: Therapeutic Potential of Bone Marrow-derived Mesenchymal Stem Cells on CCl4-induced Mouse Hepatic Injury Possibly through Matrix Metalloproteases and Differentiation into Hepatocyte-Like Cells

V. Rabani[1]*, M. Shahsavani[1], M. Gharavi[2], Z. Azdari[1], A. Piriai[1], H. Baharvand[1, 3]

1. Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Pathology, Research and Training Center of Legal Medicine Organization, Tehran, Iran
3. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran
Email: vahidehrabani@yahoo.com

Objective: To study the therapeutic effect of mesenchymal stem cells (MSC) on induced liver fibrosis in mice model and to uncover its mechanism
Materials and Methods: we infused GFP+ MSCs of male into the tail vein of female mice that received CCl4 injection biweekly to induce liver fibrosis. MSC which were derived from bone marrow obtained from femoral and tibial bones, isolated and proliferated in culture. They were characterized morphologically and by their potential of differentiation to osteo and adipo lineage. Animals were divided into 6 groups: control, CCl4 4 week, CCL4 8 week, CCl4 4week plus MSC, CCl4 8 week plus MSC, CCL4 plus opportunity to regeneration. Liver tissue was examined histopathologically and by software to quantify collagene deposition as the stage of liver fibrosis. Lipid peroxidation were quantified as a marker of liver injury level. Gene expression ratio of the collagen (type I),TIMP1,MMP-9,MMP-13,alph SMA was detected. Immunostaining were done to show homing and differentiation to hepatocyte of the cells and presentation of Hepatic stellate cells(HSC) . liver functions (serum ALT and AST) were estimated for all groups.
Results: Quantitative RT-PCR analysis showed administration of MSCs has a significant antifibrotic effect as evidenced by the significant decrease in liver colla-

gen and increase MMP13 gene expression in the CCl4/MSC group compared to the CCl4 group, 4 weeks after transplantation. The expression of αSMA (smooth muscle actin) and TIMP also reduced in CCl4/MSC group. However, this was statistically nonsignificant. Additionally, the expression of MMP9 significant increase in CCl4 treated groups; however, there was no significant change after MSC injection. The reduction in liver collagen confirmed histopathologically by quantitative analysis. Moreover, lipid peroxidation content in transplanted group decreases significantly. Immunostaining of transplanted cells showed GFP-positive cells in the liver and some of them expressed albumin or αSMA.
Conclusion: MSCs can enhance recovery of CCl4-injured mouse liver through their influence in reduction collagen deposition by possible effect on matrix metalloptoteases and their capacity to differentiate into hepatocyte-like cells

Keywords: Liver Fibrosis, CCL4, MSCs, HSCs

Ps-35: Optimization of RGD-Peptide Nano Scaffolds with Copolymrization Method for Axonla outgrowth in Differentiated hESCe-Neural Stem Cells

Rahjouei A[1]*, Zare N[1], Kiani S[1], Shahbazi E[1], Baharvand H[1, 2]

1. Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran

Email: Ali.rahjouei@gmail.com

Objective: Today, 3D scaffolds find more application in culture of various cell types with goal of artificial tissues production. RGD-peptide amphiphil is a kind of these scaffolds from self-assembly peptide family. Previous reported showed a range benefits for this scaffolds, such as induction of cell survival, facility in cell migration and more rate of cell proliferations in compare with 2D surfaces. But, RGD-peptide amphiphil purely is not supportive for neural differentiation. Therefore in this study with attention to previous reports about benefits of laminin surface for neural differentiation, we made a composite nano scaffolds from combination RGD-peptide amphiphil and laminin with copolymerization method for differentiation of "neural stem cells" derived from Human embryonic stem cells to "mature neurons".
Materials and Methods: Cell Culture, Immuno fluorescence staining for marker of mature neurons (MAPII), RT-PCR, Cell viability test (trypan Blue) and quantification of axonal growth with Software (ImageJ).
Results: Human embryonic stem cells after 5 days expansion in culture media on Matrigel surface induced to neural stem cells by induction media (1 DMEM/F12: 1 Neurobasal) supplemented with Noggin and N2. After 12 days appeared rosette and neural tube like structure. Differentiation to Neural stem cells confirmed by RT-PCR with expression of specific markers of this stage, such as nestin, sox1, Pax6 in dissociated structure and also with morphologically maturation of these cells in maturation media. Produced neural stem cells were seeded in two groups of prepared scaffolds, pure scaffolds and composite nano scaffolds respectively. Cells in pure nano scaffolds showed more proliferation and cell migration, but in this group after 2 weeks treated with differentiation observed in a circular form with viability around 68%. In composite nano scaffolds, seeded cells after passing same situation had low proliferation and cell migration but, they showed 49 times axonal outgrowth more than cells in pure nano scaffolds group. In this stage maturation of this cells confirmed MAPII immune fluorescence staining.
Conclusion: Pure RGD-peptide amphiphile nano scaffolds can support from neural stem cells migration and proliferation and in combination to laminin can support from axonal outgrowth. Therefore, with attention to previous reports in other cell types and our study for neural stem cells we can say that this peptide in combination with other peptide can produce optimize condition in a 3D nano space for differentiation of various cell types.

Keywords: RGD-Peptide Amphiphile, Human Embryonic Stem Cells, Neural Stem Cells

Ps-36: *In Vitro* Differentiation of Human Umbilical vein Mesenchymal Stem Cells into Hepatocyte-like Cells

Raoufi A[1]*, Amini[1], Fathi F[2], Azadbakht M[1], Motamedy R[1], Amini S[2]

1. Faculty of Science, RAZI University, Kermanshah, Iran
2. Kurdistan University of Medical Sciences, Sanandaj, Iran
Email: az.raoufi@gmail.com

Objective: Umbilical vein mesenchymal stem cells (UVMSC) which are recently introduced, is one of the good alternative source for mesenchymal stem cells. UVMSC have demonstrated the ability to differentiate into various cell types such as fat, bone, cartilage and neuronal cells. In this study, we have investigated whether human UVMSC are also able to differentiate into hepatocyte-like cells.
Materials and Methods: After induction, the differentiation of UVMSCs into cells expressing liver-specific genes was investigated by RT-PCR. Hepatocyte-like cells were evaluated by immunofluorescence staining (IF). Indocyanine green (ICG) was used as a test substance to evaluate hepatocyte-like cells function.
Results: The cells showed the remarkable transition from bipolar fibroblast-like morphology to round or

oval shape. The temporal gene expression pattern for a number of hepatocyte-specific genes, were detected during differentiation. The IF analysis showed that the differentiated cells were stained positively for hepatic markers. The differentiated hepatocyte-like cells were positive for ICG.
Conclusion: Based on these observations, we conclude that UVMSC retain hepatogenic potential suitable for cell therapy and transplantation against intractable liver diseases.

Keywords: Hepatocytes, Hepatice Differentiation, Umbilical Vein, Mesenchymal Stem Cells

Ps-37: Adipose - Tissue Derived Mesenchymal Stem Cell (ASC) as Alternative Source for Autologous Cell Therapy

Razmkhah M[1]*, Chenari N[1], Faghih Z[1], Khalatbari B[2], Nemati H[2], Ghaderi A[1, 2]

1. Shiraz Institute for Cancer Research, Shiraz, Iran
2. Shiraz University of Medical Sciences, Shiraz, Iran
Email: mrazmkhah@yahoo.com

Objective: Adult stem cells are undifferentiated cells that can be derived from different parts of the body including bone marrow, adipose tissue and blood. They can differentiate into many different cell types such as bone, muscle, hepatocytes, neuron and fat. One of the best-characterized adult stem cells is the mesenchymal stem cells (MSCs) that can be isolated from adipose tissue by a minimally invasive procedure. These properties have generated tremendous interest in the potential use of MSCs for regenerative medicine. The aim of this study is the isolation and characterization of adipose tissue derived mesenchymal stem cells from different sources of adipose tissue such as heart, abdomen and breast.
Materials and Methods: Fragments of adipose tissue minced and digested with collagenase. The resulted soap was centrifuged, the pellet was put on Ficoll solution and the second layer was transferred into a tube and cultured in DMEM culture medium. For characterization of ASCs, flow cytometry analysis was performed for cell surface markers including CD14, CD34, CD45, CD44, CD105 and CD166.
Results: ASCs was cultured and expanded for manyfold in sequential passages. Flow cytometry analysis of cell surface markers of the isolated cells exhibited lack of expression for CD45, CD14, CD34 and a high level expression for CD166, CD105 and CD44.
Conclusion: Immune gene transfected of autologous MSCs in patients with cancer is one of the most important applications of these cells in cell therapy. It is shown that MSCs are able to engraft in several tissues, migrate to sites of injury and differentiate into regenerating tissue. By considering the similarity between the pattern of surface markers in ASCs and bone marrow derived mesenchymal stem cells and their potential differentiation, the result of this investigation point to the important of adipose derived MSCs for both gene and cell therapy in cancer and other human disorders.

Keywords: Mesenchymal Stem Cells, Adipose Tissue, Cell Therapy

Ps-38: Kinetin Enhances *In Vitro* Development of Parthenogenetic and Nuclear Transfer Porcine Embryos

Roh S

School of Dentistry, Seoul National University, Seoul, Korea, Republic
Email: sangho@snu.ac.kr

Objective: Culture conditions affect the development of mammalian embryos in vitro. Kinetin belongs to the family of N6-substituted adenine derivates and promotes cell division, synthesis of DNA repair enzymes, superoxide dismutase activity, and ribosomal RNA transcription. We investigated the effects of kinetin on in vitro development of parthenogenetic and nuclear transfer (NT) porcine embryos.
Materials and Methods: Parthenogenetic and NT porcine embryos were cultured with or without kinetin in either BSA- or polyvinyl alcohol-containing medium for 7 days. mRNA expression of three developmentally important genes, HSP70, Glut-1, and poly[A] polymerase in NT embryos was analyzed by real-rime RT-PCR.
Results: Regardless of kinetin supplementation, the proportion of blastocysts and blastocyst cells were not significantly different in parthenogenetic embryos. However, kinetin supplementation increased expansion and hatching rates in all groups. In somatic cell NT embryos, kinetin increased the proportion of embryos developed to blastocysts from 7.5% to 15.4% in medium supplemented with PVA. However, gene expression levels of HSP70, poly[A] polymerase and Glut-1 mRNA were not significantly different in NT blastocysts.
Conclusion: The present study indicates that kinetin not only improves blastocyst expansion and cell number of parthenogenetic porcine embryos but also enhances NT porcine embryo development in a completely defined culture condition in vitro.

Keywords: Kinetin, Porcine Embryo, In Vitro Culture, Parthenogenesis, Nuclear Transfer

Ps-39: Stepwise Direct Differentiation of Human Embryonic Stem Cells into Functional Motorneurons

Shahbazi E[1, 3]*, Zare N[1], Kiani S[1], Hatami M[1], Hemmesi K[1], Rahjouei A[1], Gourabi H[2], Baharvand H[1,2]

1. Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Genetics, Royan Institute for Reproductive Biomedicine, ACAECR, Tehran, Iran
3. Department of Developmental Biology, University of Science and Culture, Tehran, Iran
Email: e.sh60@yahoo.com

Objective: Motoneurons are specialized class of neural cells essential for control of body movement and loss of these cells cause to creation of wide range of neurological disease. Embryonic stem cells (ESCs) possess promising potential for cell-based therapies of motoneuron diseases. Here, we describe a new in vitro protocol of directed differentiation of human embryonic stem cells (hESCs) into motoneuron cells.
Materials and Methods: Differentiation of neural cells was induced in DMEM/f12 media supplemented with noggin, RA and bFGF. Rosette structures were observed 12 days after induction of differentiation and then neural tube-like structures were formed at 18th day. Dissociated neural tube-like structures were treated with SHH and RA for additional 6 days in suspension culture. Sequential stages of differentiation into motorneurons were confirmed with various methods such as Immunostaining, Patch clamp recording, qRT-PCR and flowcytometry.
Results: Flowcytometry analysis of differentiated cells at neural ectoderm stage showed high expression of Nestin, Sox1, and Pax6. Differentiated neural cells have the Na, Ca currents and excitatory postsynaptic potentials (EPSPs)-like. Immunofluorescence staining of matured neural cells showed the expression of MAP2, Tuj1, HB9, islet1, choline acetyl transferase and GFAP. Analysis of Motorneuron specific markers by quantitative RT-PCR is ongoing.
Conclusion: Taking together these findings suggest that our differentiation protocol has the capacity to generate functional motorneurons.

Keywords: Motorneuron, Human Embryonic Stem Cells, Differentiation

Ps-40: Actinidin A New Collagenase for Isolation and Primary Culture of Thymic Epithelial Cells from Rat Thymus

Shirvani Farsani Z[1]*, Mostafaei A[2], Mansouri K[2], Bidmeshkipour A[1]

1. Biology, Razi University, Kermanshah, Iran
2. Immunology, Medical Kermanshah University, Kermanshah
Email: zbshirvani@gmail.com

Objective: Proteolytic enzymes, specially collagenase, are used to digest exteracellular matrix, cells isolation and primary culture. It is important to find new sources of plant or animal protease instead of bacterial or tissue collagenase. In the present research, actinidin, a plentiful protease in kiwifruit (Actinidin deliciosa), was used for isolation and culture of cells from thymic epithelial cells (TEC) from rat thymus.
Materials and Methods: Materials and Methods: The thymus was taken out. The gland was minced into small pieces and suspended in the PBS containing 1, 2, 4, 8, or 16 mg/ml actinidin for 1, 2, 3, or 4 h with gentle shaking. The cell pellet was resuspended in William's E culture medium. The cell suspensions were cultured in dishes precoated with collagen.
Results: Rat TEC was properly isolated after digestion of thymus in 4 mg/ml actinidin for 4 h at 37 °C. The isolated cells were adhered to collagen precoated dishes after washing. After 24 h of culture, the adherent cells were flattened and showed polygonal morphology with small nuclei. The viability of the cells as judged by the trypan blue test was estimated to be 90–95% in all isolations.
Conclusion: The results showed that actinidin has not toxic effect on separated cells and is a novel and suitable protease for isolation of rat TEC.

Keywords: Actinidin, Collagenase, Kiwifruit, Thymic Epithelial Cells

Ps-41: Neural Differentiation of USSC by Inhibition of GSK-3β

Vahid Dastjerdi F[1]*, Zeynali B[1], Tafreshi A.P[2], Soleimani M[3]

1. School of Biology, College of Science, University of Tehran, Tehran, Iran
2. Department of Biochemistry, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
3. Department of Hematology, Faculty of Medicine, University of Tarbiat Modares, Tehran, Iran
Email: fatemehvaheed@yahoo.com

Objective: Somatic signaling pathway. While there are large body of evidence for the involvement of this GSK-3β is a key molecule in several signaling pathways including canonical Wnt pathway in the right balance between proliferation and differentiation states of the stem cells, none is available on the newly introduced stem cell called the unrestricted stem cell (USSC).
Materials and Methods: In this study, we have therefore investigated if the activation of Wnt pathway is involved in the differentiation of USSCs into dopaminergic neurons. USSCs were isolated from umbilical cord and cultured in the presence of neural diffrentiation factors and specific GSK-3β inhibitor (BIO) for 10 days. The culture media of the control USSCs contained neural differentiation factors and the DMSO, a solvent for BIO. The expression of general neuronal marker (β-tubulin III) were examined by using flowcytometery. The activation of Wnt pathway was examined

at the expression levels of pGSK-3β and β-catenin by immunocytochemistry and western blotting.
Results: Our results showed that in cells cultured in the presence of both neural differentiation factor and BIO, the expression of general neural marker were significantly increased compared to that in the control cells. Moreover, BIO increases the experssion of pGSK-3β and β-catenin compared to that of the control, indicating that Wnt pathway is activated in these cells.
Conclusion: Altogether, these result show that the inhibition of GSK-3β in USSCs may lead to neural differentiation suggesting a role for canonical Wnt signaling pathway in differentiation of USSC stem cells.

Keywords: Wnt, GSK-3β, USSC, Neural Differentiation

Ps-42: Analysis and Production of Recombinant Peroxisomal protein (PEP)

Yari L[1]*, Ghaedi K[1], Tanhaei S[2], Rabiee F[2], Nasr-E-Esfahani M.S[3], Houshmand M[4], Zaker R[5], Baharvand H[6]

1. University of Isfahan, Stem Cells Department, Cell Sciences Research Center, Royan Institute, Isfahan, Iran
2. Stem Cells Department, Cell Sciences Research Center, Royan Institute, Isfahan, Iran
3. Royan institute, Isfahan, Iran
4. Genetic Department, National Center of Genetic Engineering and Biotechnology, Tehran, Iran
5. Biology Department, University of Isfahan, Isfahan, Iran
6. Stem Cells Department, Cell Sciences Research Center, Royan Institute, Tehran, Iran
Email: lad_ya@yahoo.com

Objective: With considering about our extended information in genome sequence field which gathered in genome data banks nowadays discovering the genes function and their products still unclear that's why study in this area sensed. Proteomics researches are one of the powerful tools for reach to this knowledge
Materials and Methods: In our study at first we prepare competent cells from Escherichia coli species named BL21 for expressing recombinant protein, then in several stages, essential condition for processing recombinant protein, of this sort; change in culture temperature, induction time and concentration of IPTG for protein expression, time and number of sonication pulses for breaks in bacterial membrane and releasing cytosolic contents ,at least change in kind and concentration of detergents for eliminate membrane debris were adjusted. All of the results step by step classified and optimized.
Results: The optimized concentration of IPTG was 0.1 mM. There was not any significant difference between the used detergents as both of them had the same effect on permeabilization of the bacterial membranes. Finally we detected the recombinant GST-PEP by SDS page analysis with both of Commasie Brilliant Blue staining (CBB) and western blot using anti GST antibody.
Conclusion: Taken together these data showed that PEP (peroxisomal protein)which containing fibronectin type III and two hydrophobic domains should be assessed by further proteomics analysis to discover it's interactions with other proteins in neural stem differentiated cells.

Keywords: Proteomics, Peroxisomal Protein(PEP), Recombinant Protein, Bacterial Competent Cell, Glutathione Sepharose

Ps-43: Plating Density Affects Growth Characteristics of Mesenchymal Stem Cells

Yazdizade A*, Khoshkhou S, Attar A, Khosravi Maharlooei M, Solhjou Zh, Pourhabibi Zarandi N, Ahrari I, Monabati A

Stem Cell, Shiraz University of Medical Sciences, Shiraz, Iran

Objective: Determination of growth characteristics of different cell types is considered as an important way to study the effect of extracellular molecules such as different culture medias in vitro. A normal growth curve consists of three phases: 1-the lag phase is the time after subculture and reseeding in which cells adapt with new conditions, 2-the log phase is the period of exponential increase in cell number and 3-the plateau phase in which cell growth is reduced due to contact inhibition and depletion of nutrients. In this study we analyzed these parameters for mesenchymal stem cells. These data will be helpful for researchers who want to study effect of different culture medias and serums efficacy.
Materials and Methods: Mesenchymal stem cells were harvested from a culture plate, counted and brought into suspensions of 1×105 cells /ml (concentration A), 3×104cells/ml (concentration B) and 1×104 cells/ml (concentration C), in 25 ml of media for each concentration. One milliliter (ml) of each was seeded in an arrow of two 24 well plates. Plates were incubated at 37°C in a humid atmosphere with 5% CO2. Media exchange was done every three days. Every day one well of each concentration was trypsinized and cells were counted with a hemocytometer. Finally growth curve was drawn and parameters were calculated.
Results: Lag time was determined as more than one day, 4 days and 4 days, with log durations of 3 days, 4 days and 4 days for concentrations A and B and C respectively. Population doubling time was calculated by the formula "(log(N1)- log(N0))/log2" and results were 1.6 , 1.12 and 1.53 for concentrations A, B and C.
Conclusion: Growth curve contained all three phases indicating that higher seeded cell number results in a lesser lag time and also a lower log duration, but lower cell concentrations lead to higher rates of proliferation.

Precongress Workshops

Bone Tissue Engineering
September 21st, 2009

Regeneration of large bone defects is being considered as challenging task by facio-mandibular and orthopedic surgeons. In these circumstances, either bone grafts or metal implants are currently being used. The inherent limitations associated with these methods have directed the attention of investigators into new technologies as bone tissue engineering which is a multidisciplinary field in which life as well as engineering sciences is involved to manufacture an appropriate bone construct.
Mesenchymal stem cells (MSCs) are considered as a suitable candidate for bone regeneration strategies owing to their ability to undergo extensive proliferation and their potential to undergo differentiation into osteoblastic, adipocytic, condrocytic cell lineages.
In this workshop, the main components involved in a bone tissue engineering process including cells (with emphasize on mesenchymal stem cells), scaffolds, the tissue engineering approaches for tissue regeneration will be trained.

Scientific Manager:

- **M. R. Baghban Eslaminejad**

Scientific Committee:

- **Hamid Nazarian**
- **Fatemeh Bagheri**
- **Sima bordbar**
- **Faeze Faghihi**

Executive Manager:

- **Hamid Nazarian**

Executive Committee:

- **Tahereh Karimi**
- **M. Ali Zare**
- **Mohammad Ghasemzadeh**
- **Shahrbanoo Jahangiri**
- **Souri Mardpour**
- **Nasrin Fallah**
- **Negar Karimi**

Office Hysteroscopy
September 21st, 2009

Nowadays , hysteroscopy has evolved considerably in favors of patient compliance , thanks to smaller instruments , but perhaps more importantly due to introduction of the vaginoscopic approach the use of speculum and tenaculum are no longer necessary Because the possibility to perform a biopsy or a small surgical intervention during the same session it is the gold standard to evaluate the uterine cavity some indication such as , some intrauterine adhesion , polyps diagnostic hysteroscopy can be done easily and without anesthesia.
The purpose of this workshop is to introduce the technique of office hysteroscopy and its indications.

Scientific Committee:

- **Dr. Micheal Kamrava (USA)**
- **Dr. Y. Shu Zhong (China)**
- **Dr. E. Shahrokh Tehraninejad (Iran)**
- **Dr. F. Ghaffari (Iran)**

Executive Committee:

- **Z. Golmohammadi**
- **F. Hosseini**

HEED & SEED
September 22nd, 2009

Hysteroscopic direct endometrial embryo delivery
Hysteroscopic sun endometrial embryo delivery
Since the traditional embryo transfer technique is blind and may contribute in part to the failure of implantation in many IVF cases, we sought to develop a procedure that 1-allows placement of embryos at the desired location in the uterus under direct visualization 2- to provide an objective visually confirmed replicable technique for embryo transfer

Scientific Committee:

- **Dr. Michael Kamrava**
- **Dr. Morzieh Shiva**

Executive Manager:

- **Zahra Ezabadi**

Hysterosonography
September 22nd, 2009

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 aims to provide an overview of the role of hysterosonography as a diagnostic procedure in infertility clinic. Target audiences will be gynecologists and radiologists.

Course includes 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.

Chairman:

- **Dr. Firoozeh Ahmadi**

Executive Manager:

- **Fatemeh Zafarani**

Hysterosalpingography
September 21st, 2009

Although hysterosalpingography has been used over the years for various indications, evaluation of infertility is the most common reason for its use today. With the recent advances in reproductive medicine, hysterosalpingography has become a relatively quick and less invasive method in the early evaluation of the infertile couple.

Accurate interpreting of HSG images will assist clinicians to minimize diagnostic error and probable complications of this procedure. The teamwork between the gynecologist specialist and radiologist is essential, and both should be present at hysterosalpingography for appropriate evaluation and diagnosis of the problem.

This workshop aims to provide an overview of the role of hysterosalpingography as a diagnostic and therapeutic procedure in infertility clinics.

The workshop will concentrate upon the indications, contraindications, technique, interpreting of normal and abnormal HSG images.

Target audiences will be gynecologists and radiologists.

Chairman:

- **Dr. Firoozeh Ahmadi**

Executive Manager:

- **Fatemeh Zafarani**

IVF Failure
September 22nd, 2009

The treatment of human infertility through the assisted reproductive technologies (ART) continues to be comparatively ineffective, despite the common practice of multiple embryo transfer.
Although the IVF-ET delivery rate is actually an improvement over the preceding years, it is obvious that the majority of IVF-ET cycle still fail, in most cases there is no apparent explanation other than failure of the implantation process. In some patients implantation failure occurs repeatedly. These latter patients continue to present unique challenges for the infertility specialist.
This course aims to provide an overview of several of the contemporary strategies used to enhance IVF-ET outcome in cases of repeated implantation failure. Target audiences will be gynecologists.
The most important strategies will be:

1. Prophylactic salpingectomy in the case of hydrosalpinges
2. Blastocyst culture and transfer
3. Assisted hatching
4. Pre implantation genetic diagnosis (aneuploidy screening)
5. Co culture methods

Scientific Committee:

- **Dr. Mahnaz Ashrafi**
- **Dr. Firouzeh Ghaffari**
- **Dr. Hamid Gourabi**
- **Dr. Poopak Eftekhari Yazdi**
- **Leili Karimian**

Executive Committee:

- **Kiandokht Kiani**
- **Masoumeh Joodmardi**

Updates on Prediction and Management of OHSS
September 21st, 2009

In-vitro fertilization treatment is a complex and relatively expensive treatment that carries significant risks of complication associated with ovarian stimulation. Despite the many approaches in ovarian stimulation protocols that have been proposed in the last decade, the risk of excessive response (hyperstimulation) to treatment with exogenous gonadotrophins remains a substantial problem.
Ovarian hyper stimulation syndrome (OHSS) is an iatrogenic complication of ovarian stimulation. It almost always presents after human chorionic gonadotrophin (HCG) administration in susceptible patients and sometimes in the very early stage of embryo implantation.(1) OHSS has been suggested to occur in two distinct forms, early and late with possibly different predisposing factors some in fertile women are more at risk for OHSS such as young women, PCOS, asiteniahabitus, neck lace sign ovary, history of OHSS, GnRH agonist protocol high serum estradiol level, multiple follicle, and multiple pregnancy.
the syndrome is characterized by fluid shift from the capillaries to the third space as a result of increased vascular permeability, possibly mediated by vascular endothelial growth factor (VEGF). The aim of this special symposium is to provide fertility professionals with an over view of OHSS, including an update on prediction and management strategies in an attempt to reduce significantly its occurrence in IVF cycles.
Review of literature on epidemiology and risk factors of OHSS, concludes that all women under going ovarian stimulation should be considered at risk of OHSS. The genetics of OHSS is an extremely interesting topic that continues to encourage a great deal of scientific work. The response to ovarian stimulation seems to be dependent on the FSH receptor (FSHR) genotype. Analyses of polymorphisms of the FSHR have demonstrated that some gene variants may be associated with extremes of response to exogenous gonadotrophines. There is the available classification of OHSS and discuss the benefits of agreeing on set categories in order to optimize patient management in routine clinical practice. There is a balanced view of the currently available preventive measures of OHSS. From taking a through medical history to the use of antagonists and in-vitro maturation of oocytes in high-risk women. A novel safe approach to prevent OHSS in the use of dopamine agonists. The mechanism by which dopamine agonists block the phosphorylaution of VEGF receptors 2. with regard to the dose of HCG given, there is a lack of robust evidence from which to draw definite conclusions. The IVF clinical outcomes, including the occurrence of OHSS, do not differ significantly when 5000 IU or 10000 IU of HCG are given to induce final oocyte maturation prior to ovum retrieval. There is some benefits of triggering ovulation with gonadotrophin releasing hormone (GnRH) agonist as compared with HCG and the importance of appropriate luteal phase supporting stimulation cycles. Finally, the IVF pregnancy outcome in women who developed OHSS should be regarded as high risk, and dedicated antenatal care should be offered.

Scientific Committee:

- **Dr. Tahereh Madani**
- **Dr. Maryam Hafezi**

Executive Manager:

- **Akram Bahmanabadi**

Ovarian Tissue Vitrification
September 22nd, 2009

Fertility preservation is becoming an important quality of life issue to the growing population of cancer survivors treated during their life fertile years. Approximately 2% of all malignant diseases occur during childhood and adolescence and cure rate can exceed 90% in this young group of patients.
Ovarian tissue cryopreservation opens new perspectives; it can be easily extracted by laparoscopy in humans without any significant delay of potentially gonadotoxic therapy. It could be used in the animal reproductive techniques too for preservation of endangered species and reduction of polymorphism. Ovarian tissue depending on the species is compromised of thousands or millions of follicles and by use of in vitro culture system; follicles could grow and mature completely.
After successful organizing of the first workshop on embryo vitrification on 26 august 2008, the 2nd vitrification workshop with main focus on ovarian tissue vitrification is going to be held on September, 22nd, 2009. This workshop aims to touch on different ovarian vitrification techniques and discussing the main questions of the necessity of clinical applications for patients.

Scientific Committee:

- **Dr. Hossein Eimani**
- **Dr. Stefania Annarita Nottola**
- **Dr. Poopak Eftekhari Yazdi**

Executive Committee:

- **Ms. Leila Sadat Tahaei**
- **Mr. Rouhollah Fathi**

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