

B-Award Winners

A-1

A comparison between anti-müllerian hormone and small antral follicle count in prediction of high responders to controlled ovarian hyperstimulation

Oskouian H, Ahmadi Sh, Aflatoonian A.

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

E-mail: HomaOskouian@gmail.com

Introduction: The aim of this study was to compare the correlation and predictive value of basal serum anti-müllerian hormone (AMH) level and small antral follicle count (2-6mm) for ovarian high response to controlled ovarian hyperstimulation (COH) in IVF cycles.

Materials and Methods: A total of 159 patients were prospectively enrolled. Basal serum AMH and small AFC (2-6mm) were measured before starting ovarian stimulation.

Results: AFC (2-6mm) and AMH were the most significant predictors of ovarian high response with similar predictive accuracy on multiple regression analysis and had the largest area under curve (AUC= 0.961 and AUC= 0.922 respectively). The sensitivity and specificity for prediction of ovarian high response were 89% and 92% for small AFC and 93% and 78% for AMH at the cutoff value of ≥ 16 and ≥ 34.5 M/mL, respectively. AMH was the only significant predictor of embryo quality.

Conclusion: AMH and small AFC had similar predictive accuracy but, because of the intercycle variability and operator-dependency of small AFC, the measurement of AMH level is the most useful test for prediction of ovarian high response.

Key words: Anti-müllerian hormone, Antral follicular count, Ovarian hyperstimulation syndrome.

A-2

Generation of motor neurons by co-culture of retinoic acid-pretreated embryonic stem cells with chicken notochords

Anjomshoa M^{1, 2}, Karbalaie Kh¹, Mardani M², Razavi Sh², Tanhaei S¹, Nasr Esfahani MH¹, Baharvand H^{3, 4}.

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

2 Department of Anatomical Sciences, School of Medicine, Esfahan University of Medical Sciences, Esfahan, Iran.

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

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

E-mail: Baharvand50@yahoo.com

Introduction: Understanding neuroectoderm formation and its subsequent diversification to functional neural subtypes remains elusive. We have shown here for the first time that embryonic stem cells (ESCs) can differentiate into neurons and motor neurons (MNs) by using a co-culture embryonic notochord model in vitro.

Materials and Methods: Mouse ESCs were induced to form neural precursors via timed exposure to retinoic acid (RA) using the 4-/4+ RA protocol. These cells were then co-cultured with alginate bead-encapsulated notochords isolated from Hamburger and Hamilton stage 6-10 chick embryos. The use of notochord alone was not able to induce neural differentiation from ESCs and therefore, notochord does not possess neural inducing activity.

Results: The most successful neuronal cells and MN differentiation was only observed following the co-culture of RA-pretreated ESCs with notochord. This resulted in a significantly greater number of cells expressing microtubule associated protein 2 (MAP2), HB9, choline acetyltransferase (ChAT) and MN-specific genes.

Conclusion: While further characterization of these differentiated cells will be essential before transplantation studies commence, these data illustrate the effectiveness of embryonic notochord co-culture, in providing valuable molecular cues for directed differentiation of ESCs towards an MN lineage.

Key words: Co-culture, Embryonic stem cells, Motor neurons, Neuronal induction, Notochord, Retinoic acid.

A-3

Characterisation of the surface proteome of oviductal epithelial cells in different phases of the reproductive cycle

Ritchie AI, Pewsey EM, Staves J, Bruce Ch, Elliott S, Fazeli A.

Academic Unit of Reproductive and Developmental Medicine, University of Sheffield, Level 4, Jessop Wing, S10 2SF Sheffield, UK.

E-mail: mda03air@sheffield.ac.uk

Introduction: The mammalian oviduct is the site of crucial events leading to fertilization and the

establishment of a successful pregnancy. The surface of the oviductal epithelial cells (OEC) is the main site of contact with both gametes during these events. Therefore, it is hypothesized that changes in the oviductal epithelial cells surface proteome taking place during follicular and luteal phases of the cycle may play important role in events leading to conception and embryo development. This work is intended to identify these changes and further understand how the oviductal environment impacts on the process of fertilization.

Materials and Methods: Oviductal epithelial cells from pig oviducts in follicular and luteal stages were isolated and their surface proteins were marked with the molecule biotin. We used a combination of two-dimensional gel electrophoresis and liquid chromatography-tandem mass spectrometry to identify differences in the cell surface proteome during the different stages of the reproductive cycle.

Results: We found 15 upregulated and 25 downregulated proteins during the follicular relative to luteal phase. We identified eleven of the upregulated and seventeen of the downregulated proteins. The findings show that the oviductal cell proteome is altered during the reproductive cycle. Work is ongoing to verify the changes observed in this study of the oviductal cell-surface during the reproductive cycle, through Western blotting.

Conclusion: Our results demonstrate the dynamic nature of the oviductal environment at the cell surface. This is important because the oviductal cell surface is the site of interaction with gametes and embryos. We are currently in the process of validating our 2D gel electrophoresis findings using Western blotting, and conducting bioinformatic analysis to identify the role of each protein in the reproductive tract and specifically at the surface of the cell. This will lead to a better understanding of maternal interaction with gametes and embryos which will have implications in the development of effective treatments for infertility and the field of assisted reproduction.

Key words: Surface proteome, Oviductal epithelial cells, Reproductive cycle.

A-4

Study of minocycline effect on apoptosis and expression of proapoptotic proteins in mouse spermatogenic cells

Khorsandi LS¹, Hashemitabar M¹, Orazizadeh M¹, Albughobeish N².

1 Cell and Molecular Research Center, Ahvaz Jundishapour University of Medical Sciences, Ahvaz, Iran.

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

E-mail: layasadat@yahoo.com

Introduction: Abnormality accelerated apoptosis of germ cells leads to an imbalance of cell proliferation and death, resulting in spermatogenic impairment. Some studies have shown antiapoptotic effect of minocycline. In this study inhibitory effect of minocycline on apoptosis and expression of Bax and FasL (Fas ligand) proteins in spermatogenic cells arising from dexamethasone (Dex) have been evaluated.

Materials and Methods: 32 adult male (6-8 weeks) mice were divided in 4 groups. First and second groups received 100 mg/kg minocycline (i.p.) and 7 mg/kg Dex (i.p.) respectively for a week. Third group was given 100 mg/kg minocycline+7 mg/kg Dex at the same time for a week. Control group received only saline. Then the mice were sacrificed, and the testes were removed for immunohistochemistry and TUNEL studies.

Results: Apoptotic index of germ cells and expression of Bax and FasL proteins showed significant increase in Dex group ($p<0.01$). Minocycline+ Dex group showed significant reduction in Bax and FasL expression in comparison to Dex group ($p<0.05$). Apoptotic index of germ cells were also significantly decreased in this group compared with Dex treated mice ($p<0.01$).

Conclusion: Minocycline may have inhibitory effect on apoptosis and expression of proapoptotic proteins in testicular germ cells. The application of minocycline may serve beneficial effects on fertility.

Key words: Apoptosis, Spermatogenesis, Minocycline.

A-5

An assessment of lifestyle modification versus medical treatment with clomiphene citrate, metformin, and clomiphene citrate-metformin in patients with polycystic ovary syndrome (PCO)

Javedani M, Karimzadeh MA.

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

E-mail: javedani46@yahoo.com

Introduction: Polycystic ovary syndrome is a description for a broad spectrum of clinical and morphological findings on an endocrine dysfunction in women at reproductive age, especially androgen production and metabolic abnormality. The aim of the present study is to compare the effect of Clomiphene citrate, metformin, and lifestyle modification on treatment of patients with PCOs.

Materials and Methods: A prospective randomized double blind study was performed among 343 overweight, infertile women with PCOs. They were assigned to 4 groups: Clomiphene group (n=90), metformin group (n=90), clomiphene+metformin (n=88) and life style modification (n=75). The patients in each group received standardized dietary and exercise. The primary outcome variables were; change in menstrual cycle, waist circumference measurements, endocrine parameters and lipid profile. The main secondary outcome variable was clinical pregnancy rate.

Results: All of the patients showed improvements in menstrual frequency ($p=0.38$). The clinical pregnancy rate was 12.2% in clomiphene group, 14.4% in metformin group, 14.8% in clomiphene + metformin group, and 20% in lifestyle modification group ($p=0.56$). Lifestyle modification group achieved a significant reduction in waist circumference, total androgen, and lipid profile.

Conclusion: Lifestyle modification improves the lipid profile in PCOs patients. Therefore, lifestyle modification may be used as the first line of ovulation induction in PCOs patients.

Key words: Polycystic ovary syndrome, Clomiphene, Metformin, Lifestyle modification, Ovulation induction.

A-6

The novel finding of high density of activated mast cells in endometrial polyps

Al-Jefout M^{1,2,3}, Black K², Schulke L¹, Berbic M¹, Luscombe G¹, Tokushige N¹, Manconi F¹, Markham R¹, Fraser IS^{1,2}.

1 Department of Obstetrics and Gynecology, Queen Elizabeth II Research Institute for Mothers and Infants, University of Sydney, Sydney, 2006, Australia

2 Royal Prince Alfred Hospital, Missenden Road, Camperdown, NSW 2050, Australia.

3 Department of Obstetrics and Gynecology, Medical Faculty, Mutah University, Jordan.

E-mail: drmoamar@yahoo.co.uk

Introduction: Endometrial polyps (EP) are common, mostly benign lesions that are pedunculated or sessile and frequently solitary. They can be found at any age in the reproductive or postmenopausal phases of life. Using sonogram, sonohysterography or hysteroscopy, EP has been reported in 50% of women with postmenopausal bleeding, in 13% of perimenopausal women with abnormal uterine bleeding, and in 26.5% women with unexplained infertility.

Materials and Methods: We performed an immunohistochemical study on polypectomy and curettage specimens from women with EP (n=20) and from women with normal endometrium (n=19) using antibody against mast cell tryptase (Dako, M7052) at 1/100 dilution. Menstrual cycle dating of the endometrium was undertaken by experienced gynaecological pathologists at Royal Prince Alfred Hospital, (Sydney) using standard criteria.

Results: In women with EP dramatically elevated densities of activated mast cells expressing tryptase (aMCt) were identified in polyp tissues (113.0 per $\text{mm}^2 \pm 98.5$ (mean \pm SD) compared with adjacent endometrium (37.9 \pm 44.7 per mm^2) ($p<0.001$). The density of aMCt in normal endometrium was 15.9 \pm 36.6 per mm^2 . There were no significant differences in aMCt densities in EP at different phases of the menstrual cycle. However, mean activated mast cell density in endometrium adjacent to polyps was found to be highest in the menstrual phase (69.3 \pm 64.3) and lowest in the secretory phase (28.5 \pm 24.3) ($p<0.005$). When activated mast cell density in polyps was compared between the three phases of menstrual cycle using a Kruskal-Wallis chi-square, there was no statistically significant difference between the groups. When the polyp data were examined for a linear trend across the cycle using Spearman's correlation, there was still no significant relationship. Furthermore, when activated mast cell density in the adjacent endometrium was compared between the three phases of menstrual cycle using a Kruskal-Wallis chi-square, there was also no statistically significant difference between the groups. However, when the data on activated mast cell density across the phases in adjacent endometrium were investigated for a linear trend using a Spearman's correlation, there was a significant negative correlation ($r_s=-0.25$, $p=0.045$ or $p<0.05$), with highest densities in menstrual phase.

Conclusion: This unexpected but consistent observation in endometrial polyps suggests that activated mast cells may play important roles in general polyp biology in many tissues. They may play initiating roles in polyp growth and contribute to continuing development. They may also secrete a range of active molecules which may contribute to symptoms such as surface bleeding. This observation opens a new field of investigation in polyp biology and may provide therapeutic leads which can prevent polyp growth or recurrence.

Key words: Novel findings, Activated mast cells, Endometrial polyps.

A-7

Transplantation of primed or unprimed mouse embryonic stem cell-derived neural precursor cells improves cognitive function in Alzheimerian Rats

Homayouni Moghadam F^{1,2,3}, Alaie H², Karbalaie Kh¹, Tanhaei S¹, Nasr Esfahani MH¹, Baharvand H^{4,5}.

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

2 Department of Physiology, Medical Faculty, Isfahan University of Medical Science, Isfahan, Iran.

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

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

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

E-mail: Baharvand50@yahoo.com

Introduction: Alzheimer's disease (AD) is a neurodegenerative disorder that is characterized by progressive and irreversible decline of memory. Neuropathological features include the progressive degeneration of cholinergic neurons in the forebrain cholinergic projection system especially nucleus basalis of Meynert (nbM). New cell therapeutic approaches for the replacement of degenerated cells are being researched. The aim of this study was to investigate the production of cholinergic neurons from mouse embryonic stem cells (ESCs) and potential for utilizing ESC-derived neuronal precursor cells (NPCs) and primed NPCs (PNPCs) for cell restorative therapy in a rodent model of AD.

Materials and Methods: NPCs were produced by growth factor-mediated selection under serum free conditions and differentiated better into cholinergic neurons when NPCs primed with Shh (~22%) in comparison with different cholinergic promoting factors. Behavioral assessment of unilateral nbM

ibotenic acid-lesioned rats by Morris water maze and spatial probe test revealed a significant behavioral improvement in memory deficits following transplantation with NPCs and/or PNPCs.

Results: Immunohistochemical analysis revealed that the majority (~70%) of the NPCs and/or PNPCs retained neuronal phenotype and ~40% of them had a cholinergic cell phenotype following transplantation with no tumor formation, indicating that these may be safe for transplantation.

Conclusion: This experimental study has important implications as it suggests that the transplantation of mouse ESC-derived NPCs and/or following commitment to a cholinergic cell phenotype can promote behavioral recovery in a rodent model of AD.

Key words: Alzheimer's disease, Behavior; Cholinergic neurons, Differentiation, Neuronal precursor cells, Transplantation.

A-8

Survival rate of preantral follicles derived from vitrified neonate mouse ovarian tissue by cryotop and conventional methods

Mamzoojia SS³, Eimani H^{1,2}, Soleimani MM³, Abnosi MH³, Rezazadeh Valojerdi M^{1,4}, Eftekhari Yazdi P¹, Shahverdi A¹, Guorabi H¹.

1 Department of Embryology, Reproductive Medicine Center, Royan Institute, Tehran, Iran.

2 Department of Anatomy, Faculty of Medicine, Baqiatallah (a.s.) University of Medical Sciences, Tehran, Iran.

3 Department of Biology, Faculty of Basic Science, Arak University, Arak, Iran.

4 Department of Anatomy, Faculty of Medicine, Tarbiat Modarres University, Tehran, Iran.

E-mail: Eimanih@yahoo.com.

Introduction: The aim of this study was to investigate the growth and survival rate of preantral follicles isolated from vitrified ovarian tissue by Cryotop and conventional methods.

Materials and Methods: The ovaries of 14-day-old mice were separated and divided into four groups as following: 1/ Cryotop group (vitrified by Cryotop), 2/ Conventional or CV group (vitrified by conventional straw), 3/ toxicity test group and 4/ control group. After warming the vitrified ovaries, isolated preantral follicles from four groups were cultured for 4 days to compare survival rate and follicular growth between above-mentioned groups.

Results: Survival rate (97.3%) in toxicity test group alike the control group (98.7%) were

significantly higher ($p < 0.05$) than the Cryotop (92.7%) and CV (47.7%) groups. Increase in follicle diameters after 4 days in Cryotop and CV groups was significantly lower ($p < 0.05$) than the control and toxicity test groups, but growth and survival rate of follicles in Cryotop group was significantly higher ($p < 0.05$) than the CV group.

Conclusion: These results demonstrated that ovarian tissue vitrification by Cryotop highly preserves the viability rate of preantral follicles.

Key words: Vitrification, Ovarian tissue, Preantral follicles, Cryotop.

A-9

Hysteroscopic polypectomy without cycle cancellation in IVF cycle

Ghaffari F¹, Madani T^{1,2}, Kiani K¹, Hosseini F¹.

1 Department of Endocrinology and Female Infertility, Reproductive Medicine Research Centre, Royan institute, ACECR, Tehran, Iran.

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

E-mail: f.ghaffari@royaninstitute.org

Introduction: Endometrial polyps destroy the endometrial texture and play an important role in the implantation failure and miscarriage. There is no consensus about the management of patients diagnosed with endometrial polyp in IVF cycles. The purpose of this study was to do a hysteroscopic polypectomy during stimulation period in the ART cycle.

Materials and Methods: In this study, nine patients who underwent ART cycles were diagnosed to have endometrial polyps less than 1.5 cm by transvaginal ultrasonography. Eight patients were treated by GnRH Agonist long protocol and one patient was the recipient of the egg donation cycle. In All patients, polyp resection was performed through hysteroscopic polypectomy under general anesthesia after obtaining the informed consent. Polypectomy was done during ovarian stimulation in ART cycle, along with hormone replacement therapy in the recipient of egg donation cycle. The interval between polyp resection and embryo transfer was two to sixteen days.

Results: As a result, four patients achieved pregnancy (two twins, two singletons), four patients were unsuccessful, and one pregnancy was terminated because of a blighted ovum. All of the successful pregnancies are still ongoing.

Conclusion: There is a dilemma regarding the management of patients diagnosed with endometrial polyp in an ART cycle. If

polypectomy before embryo transfer in the same IVF cycle is proven to be safe, then embryo transfer without canceling the cycle would be done. This study has been done in nine patients; However further studies with more patients are required to confirm these findings.

Key words: Polyp, Hysteroscopy, IVF.

A-10

Molecular study of internal apoptotic pathway BAX and BCL2 genes and mitochondrial genome in idiopathic repeated pregnancy loss

Syedhassani SM^{1,2}, Houshmand M¹, Kalantar SM², Modabber G¹, Hadipur F³, Rasti A², Afatoonian A².

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

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

3 Special Medical Center, Tehran, Iran.

E-mail: Sayedhassani@yahoo.com

Introduction: 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. 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: 376 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 were suffered from idiopathic RPL.

1- Four multiplex PCR were done on each sample for detection of mitochondrial deletions

2- Mitochondrial D-loop part consisting of the hyper variable regions was analyzed by PCR-sequencing method.

3- Bax gene was evaluated by PCR-sequencing method for promoter region and exones.

4- Bcl2 gene was evaluated by PCR-sequencing method for promoter region and PCR-SSCP for the exones.

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 (among 89 cases; 7C in 43, 8C in 34, 9C in 8 and $\geq 10C$ in 4 females)

3- Some sequence alterations identified in D-loop region of cases, such as: T to G at nt.125, A to G at nt.73, 93 and 200, G to A at nt.207, T to G at nt.125, T to C at nt.146 and 152; that will be described as mtDNA haplogroups or novel nucleotide variants.

4- Change of A to G in promoter region of Bax gene was seen at nt. -55.

Conclusion:

1- Because oxidative stress is one of the important cause of mtDNA deletions, we suggest that this phenomenon is not 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- We believe that mutation in Bax gene will lead to early apoptosis.

4- The results can be used in assessment of RPL and probability of interventional treatment for improving of fertilization in ART methods.

Key words: BAX gene, BCL2 gene, Mitochondrial genome, Idiopathic repeated pregnancy loss.

A-11

The effect of selenium on Reactive Oxygen Species concentration of cultured prenatal follicle from vitrified mouse ovaries using conventional and direct cover technique

Abedelahi A¹, Salehnia M¹, Alameh AA².

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

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

E-mail: abedanatomy@yahoo.com

Introduction: Many in vitro culture and maturation of preantral follicles systems have been developed for studying the oogenesis, folliculogenesis and oocytes-somatic cell interactions and it has shown that the isolated

follicles were in vitro cultured and matured successfully. During in vitro culture, the cells are maintained under higher concentrations of O₂ and the Reactive oxygen species (ROS) are produced continuously and may cause impairment of ovarian tissue. To avoidance of oxidative of oxidative stress antioxidants have been used. Selenium is an essential trace element and as a component of enzymatic antioxidant that protect the cells from oxidative damages.

Materials and Methods: Prenatal follicles isolated from mice ovaries (12 to 14-day-old NMRI) and cultured in TCM 199 medium with different concentrations (0, 5, 10, 15 ng/ml) of SS and supplemented with 3 mg/ml bovine serum albumin (BSA) or 5% Fetal Bovine Serum (FBS). At day 12 of culturing the ovulation was induced by addition of 1.5 IU/ml human chorionic gonadotropin and evaluated by viability, diameter, antrum formation of follicles and the diameter and released MII oocytes. After collection of MII oocytes and their insemination with capacitated sperms, the development of the embryos was assessed up to 120 hours. After 0, 12, 24, 48, 72 and 96 h culture of vitrified and non-vitrified isolated follicles in different concentrations of SS and supplemented with FBS the ROS production were analyzed by spectrofluorometry and confocal microscopic studies. Also the total antioxidant capacity (TAC) and glutathione peroxides (GPx) levels were evaluated in the same groups in the same groups. The comparison between two groups of vitrifications (conventional and direct cover vitrification) was done. The survival rates, the mean diameter of follicles and the percentage of MII oocyte derived from follicles were cultured in FBS supplemented groups containing 10 ng/ml SS (90.83%, 199.84±15.58µm and 33.08% respectively) were significantly higher than other groups (p<0.001).

Results: The ROS levels decreased in follicles from non-vitrified and vitrified cultured with 10ng/ml SS than the controls (p<0.001) but the increases in the level of TAC and GPx activity were observed in previous groups than their controls (p<0.001). However there were significant differences between the ROS levels, TAC and GPx activity in vitrified than non-vitrified groups at the beginning of the culturing also the confocal microscopic images approved these results.

Conclusion: Our results demonstrated that the culture of preantral follicles in the FBS medium containing 10 ng/ml SS improved their maturation and growth. This effect of SS may be mediated by decrease in ROS levels and/or increase in the

follicular TAC levels and GPx activity during culture periods.

Key words: *In vitro* maturation, Oocyte, Preantral follicles, Sodium selenium, Serum free media.

A-12

Development and characterisation on an *in vitro* model of persistent *Chlamydia trachomatis* infection using physiological levels of IFN- γ found in semen

Kokab A, Jennings R, Eley A, Cross N, Pacey A.
Academic Unit of Reproductive and Developmental Medicine, School of Medicine and Biomedical Sciences, University of Sheffield, United Kingdom.

E-mail: A.Kokab@sheffield.ac.uk

Introduction: *Chlamydia trachomatis* is an obligate intracellular bacterium and is the commonest sexually transmitted disease of humans. The disease is complicated by persisting infections which may lead to pelvic inflammatory disease, ectopic pregnancy, altered semen quality and infertility. The immune response against *C. trachomatis* strongly depends on the inflammatory cytokine interferon- γ (IFN- γ) however this it self may induce aberrant and persistent forms. The aim of this study was to develop and characterise a model of *C. trachomatis* persistence *in vitro* and to identify a gene signature that may indicate the presence of persistent infections.

Materials and Methods: Using HEp-2 cells, we assessed the morphology and infectivity of *C. trachomatis* inclusions +/- physiological levels of IFN- γ (0.5ng/ml) using confocal immunofluorescence microscopy. In addition, the expression levels of *C. trachomatis* transcripts (AmiA, DnaK, FtsW, FtsK, ct604, ct755, Omp1) +/-INF- γ were determined at various time-points post-infection by Real-time PCR.

Results: IFN- γ treatment delayed the *Chlamydial* growth cycle, induced aberrant inclusion bodies and failed to eliminate infectivity. In untreated cultures, ct604 and ct755 expression levels decreased markedly between 72 hours and 96 hours post-infection, relative to 16S rRNA. Conversely in IFN- γ treated cultures, these transcripts remained elevated.

Conclusion: Using this model, persistent infections of *C. trachomatis* can be induced in Hep2 cells by treatment with IFN- γ , but these forms remain infectious. Persistent forms of *C. trachomatis* are associated with a gene expression signature of elevated ct604 and ct755, consistent

with failure to complete the natural *Chlamydial* life cycle.

Key words: *Chlamydia*, Infertility, IFN- γ -induced persistence.

A-13

Evaluation of the relationship between plasma concentrations of en- and zuclomiphene and induction of ovulation in anovulatory women being treated with clomiphene citrate

Ghobadi C, Amer S, Lashen H, Lennard MS, Ledger WL, Rostami-Hodjegan A.

Academic Unit of Clinical Pharmacology, University of Sheffield, Sheffield, UK.

Academic Unit of Reproductive and Developmental Medicine, University of Sheffield, Sheffield, UK.

E-mail: C.Ghobadi@sheffield.ac.uk

Introduction: To investigate the relationship between the plasma concentrations of clomiphene citrate (CC) isomers zu- (Zu) and enclomiphene (En), and ovulation outcome.

Materials and Methods: During a Prospective, cohort study in Reproductive medicine and fertility center in a university teaching hospital, United Kingdom Forty-two women with World Health Organization type 2 infertility were selected. The clinical and biochemical features of patients who were about to start CC for induction of ovulation were recorded. Plasma concentration of Zu and En were monitored at three points (days 2, 8, and 21) throughout the treatment cycle(s).

Results: Thirty-nine patients completed the study. Both En and Zu accumulated throughout treatment. Among the 36 responders, there was no statistically significant relationship between the clinical and biochemical characteristics of the patients, En or Zu concentrations, and the dose required to induce ovulation. Moreover, the Zu and En concentrations were not different in the three patients who failed to respond.

Conclusion: The concentrations of En and Zu in plasma, on their own or in combination with other covariates (e.g., weight, body mass index, free androgen index), are not a predictor of the ovulation response to CC or of the dose requirement. Further studies are needed to explore the role of additional covariates, including the presence of active metabolites, and the balance of the effects of En and Zu.

Key words: *Clomiphene citrate, Concentration-response, Clomiphene isomers, Ovulatory response, Infertility treatment, Normogonadotropic anovulatory infertility.*

A-14

Association of p53 polymorphism with ICSI/IVF failure and recurrent pregnancy loss

Ayazi Rouzbahani M, Dehghani Firouzabadi R, Ghasemi N, Tabibnejad N.

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

E-mail: ayaziroz@yahoo.com

Introduction: The p53 tumour suppressor gene is a well-known factor regulating apoptosis in a wide variety of cells. Alterations in the p53 gene are among the most common genetic changes in human cancers. Several polymorphisms of the p53 tumour suppressor gene have been associated with recurrent pregnancy loss.

To evaluate the association of polymorphisms p53 codon 72 with the response to in-vitro fertilization treatment and occurrence of repeated miscarriages.

Materials and Methods: The homozygous and heterozygous genotypes and allelic frequencies of Arg and Pro p53 at codon 72 were identified by using PCR-RFLP technique in 70 infertile women with more than two IVF failures. Each comparison was made with 97 women experiencing recurrent pregnancy loss (RPL) and 32 fertile women each with at least two healthy children as the control group.

Results: The frequency of homozygous Pro/Pro genotypes was found significantly higher among the women with RPL than the other two groups ($p=0.041$). Whereas, Arg/Arg genotype was significantly different in the recurrent implantation failure (RIF) group ($p = 0.005$).

Conclusion: It is concluded that P53 codon 72 polymorphism may serve as a susceptible factor affecting the chances of RPL and RIF.

Key words: P53 tumour suppressor gene polymorphism, IVF failure, Recurrent pregnancy loss.

A-15

Vascular Endothelial Growth Factor Gene Polymorphism and Ovarian hyperstimulation syndrome

Ahmadi Sh, Oskouian H, Ghasemi N, Dehghani Firouzabadi R.

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

E-mail: AHMADISHAHNAZ2005@yahoo.com

Introduction: Ovarian hyperstimulation syndrome (OHSS) is one of the most important complications of assisted reproduction treatment. The pathophysiology of OHSS remains to be fully elucidated. Many substances and more recently, vascular endothelial growth factor (VEGF) have been suggested to be involved in the pathogenesis of OHSS. VEGF is a member of a family of heparin-binding proteins that act directly on endothelial cells to induce proliferation and angiogenesis. In vivo, VEGF is a powerful mediator of vessel permeability. Increased vascular permeability mediated by VEGF has been implicated in the sudden increase in capillary permeability, then it is logical to examine the relationship between the VEGF polymorphism and OHSS. This case-control project was evaluated potential association between OHSS and polymorphism 460 of VEGF gene.

Materials and Methods: Seventy five OHSS patients and 85 normoresponse patients were enrolled in this study. Polymerase chain reaction-restriction fragment length polymorphism analysis was used to analyse the VEGF 460 genotype of OHSS patients and normoresponder controls.

Results: The frequency of homozygosity of the VEGF 460 gene was significantly higher among women with mild to moderate OHSS.

Conclusion: Homozygosity of the VEGF 460 gene polymorphism may serve as a susceptibility factor affecting OHSS.

Key words: Ovarian hyperstimulation syndrome, Vascular endothelial factor.