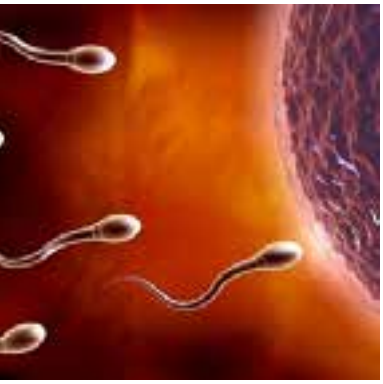
Poster Presentations

Embryology 2017





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Non-invasive mass spectrometric viability assessment of *in vitro* fertilized embryos using the alpha-1 chain of human haptoglobin

Gergely Montskó, Krisztina Gödöny, Ákos Várnagy, József Bódis and **Gábor L Kovács**
University of Pécs, Hungary

Infertility nowadays is a growing health issue in the developed world meaning that every year more and more couples visit an assisted reproduction (ART) centre. However, the success rate of the process is stagnating at about 30%. An effort is made worldwide to find new additional indicators of embryo viability to implement the routinely used morphological evaluation. Spent embryo culture medium samples (n=201) were measured using liquid chromatography coupled mass spectrometry in a series of retrospective, blind experiments. No sample preparation was made, 10 µl of sample was directly injected into the instrument after the addition of internal standard solution. A protein marker was found which significantly ($p<0.001$) differed in quantity between the samples of embryos which did, or did not implant. This protein was identified as the alpha-1 chain of human haptoglobin molecule. A significant correlation ($p<0.001$) was also found when comparing the clinical outcome (clinical pregnancy or no pregnancy) and the outcome predicted by the measurements. The haptoglobin fragment quantitation serves as an additional tool along the process of morphological evaluation. The blind, retrospective results provided a positive predictive value of more than 50%. The negative predictive value of the analysis was 100%, there were no embryos which were diagnosed as "viable" but resulted in clinical pregnancy. The results provided a contra selection tool, screening the embryos with good morphological aspects, but no implantation potential.

e: montsko.gergely@pte.hu

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Incidence of embryonic aneuploidy in different age groups of Saudi population undergoing PGS-ICSI

Murid Javed, Othman Abdulrazzak, Fatima Abdelraouf, Tagwa Saad, Sahar Bengawi, Suleiman Najashi and **Hamad Sufyan**
Thuriah Medical Center, Saudi Arabia

Introduction: Preimplantation Genetic Screening (PGS) is becoming more frequently employed for detection of aneuploid embryos to prevent transmission of genetic defects. A few cells are micro-surgically removed from the embryo to analyze the DNA, thus selecting embryos with the highest potential for implantation to optimize a woman's chance of healthy live birth and reduce the risk of miscarriage due to chromosomal aneuploidy. The objective of this study was to determine incidence of embryonic aneuploidy in different age groups of Saudi patients undergoing ICSI-PGS cycles from Jan to Dec 2016 at Thuriah Medical Center, Riyadh, Saudi Arabia.

Materials & Methods: Data from 248 patients undergoing ICSI-PGS were analyzed. The ovarian stimulation, oocyte retrieval, ICSI, PGS, culture and transfer were performed by standard protocols. All sperm samples were from male partners; 242 fresh ejaculates, 4 frozen-thawed Micro-TESE and 2 TESA. The embryo biopsies were performed on day-3 (Fig 1) and analyzed using fluorescence in-situ hybridization with probes for chromosomes 13, 18, 21, X and Y. The total embryos analyzed were 1055 and grouped into normal, abnormal, mosaic and undiagnosed. The patients were grouped into following age categories; <35, 35-37, 38-40, 41-42 and 43-48 yrs. The statistical analyses were performed by SPSS.

Results: The percentages of euploid embryos were; 43, 46, 42, 24 and 28 in <35, 35-37, 38-40, 41-42 and 43-48 yr females, respectively (Table 1). The percentages of aneuploidy embryos were; 36, 32, 37, 53 and 54 in these study groups, respectively. The percentages of normal embryos decreased and percentages of abnormal embryos increased significantly in females >41 yrs of age. The percentage of mosaic embryos was significantly higher in 43-48 yr females. The pregnancy rate was 28, 33, 22, 0 and 25% in <35, 35-37, 38-40, 41-42 and 43-48 yrs age groups, respectively. In an earlier study of Saudi population conducted in 2013, the abnormal embryos constituted 36 % in women with an average age of 34.9 yrs (1). In our study the % abnormal embryos in < 35 years age group is similar; however, it increased significantly in women ≥ 41 yrs. Such data is not available in the previous report.

Conclusion: The embryonic aneuploidy rate is similar until age 40, however, it increases significantly in embryos from 41 years or older women.

Speaker Biography

Dr. Javed is Director of ART Laboratories at Thuriah Medical Center, Riyadh, Saudi Arabia. He has been certified by Canadian Fertility and Andrology Society. He is member of Practice Committee and Chair of Certification Committee of American College of Embryology, USA. He is member of many professional societies including American Society for Reproductive Medicine, Canadian Fertility and Andrology Society and European society for Human Reproduction and Embryology.His professional carrier started after obtaining Doctor of Veterinary Medicine Degree in 1981. He earned MS in Reproductive Physiology in 1984. His initial research experiments were in Embryo Physiology of research animals. This was the time when this technology was just beginning in human. In 1986, his curiosity for further knowledge, took him to Washington State University, USA for PhD in Embryo Physiology. He earned PhD in 1990. He had the opportunity to earn 2 post doctorate fellowships; first at Kyoto University, Japan and second at University of Georgia, Athens, USA. At these institutions, he conducted research on *in vitro* fertilization, embryo culture and embryo vitrification.

e: murid.javed@thuriah.com.sa

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Generation of cloned adult muscular pigs with myostatin gene mutation by genetic engineering

Xi-Jun Yin[1], Jin-Dan Kang[1], Seokjoong Kim[2], Long Jin[1], Qing Guo[1], Xiaoxu Xing[1], Meifu Xuan[1], Guanglei Zhang[1], Qirong Luo[1], Yong Soo Kim[3], Chengdu Cui[1], Wenxue Li[1], Zhengyun Cui[1] and **Jin-Soo Kim[4]**
[1]Yanbian University, China
[2]ToolGen, Inc., South Korea
[3]University of Hawaii, USA
[4]Institute for Basic Science, South Korea

Because skeletal muscle is the most economically valuable tissue in meat-producing animals, enhancing muscle growth in these species may enhance the efficiency of meat production. Skeletal muscle mass is negatively regulated by myostatin (MSTN), and non-functional mutations of the MSTN gene in various animal species have led to dramatic hypermuscularity. This study was designed to assess the characteristics of male MSTN-knockout (KO) pigs. A transcription activator-like effector nuclease (TALEN) pair targeting exon 1 of the swine MSTN gene was constructed and used to transfect porcine fetal fibroblasts (PFFs). We obtained a cell line that consisting of a 2-bp deletion in one allele and a 4-bp deletion in the other allele, was used as a donor to generate cloned pigs via SCNT, and delivered 18 live piglets. They developed and grown normally to sexual maturity. These MSTN-KO boars grew normally to adulthood and showed visually-clear hypermuscular characteristics, increased carcass dressing percentage and loin eye size, and decreased in backfat thickness. These pigs may show greater meat production, as well as being used in animal models of human diseases.

Speaker Biography

Xi-Jun Yin is working as the Director of Jilin Provincial Transgenic Animal and Embryo Engineering Laboratory at Yanbian University. His research goal is to increase reproductive efficiency of swine and to expand the genetic potential present in pig embryos. Recently, his research team successfully produced myostatin gene knockout double-muscled adult pigs.

e: yinxj33@msn.com

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Evaluation of *in vitro* fertilization outcomes using interleukin-8 in culture medium of human preimplantation embryos

Guanyou Huang
Guizhou Medical University, China

Currently, the morphological method is mainly used for selecting embryos which are to be transferred, but this method is relatively poor for prediction of successful implantation. In recent years, non-invasive observation of embryo development has been considered as a better method of embryo viability assessment. The assessment of embryo quality and prediction of in vitro fertilization (IVF) outcome with cytokines in the culture media (EM) of human pre-implantation embryos (HPE) has been explored for years. Researchers have detected tumor necrosis factor alpha (TNF alpha) and leukemia inhibitory factor (LIF) in EM of HPE, and have raised the possibility that LIF could have a function as a factor required for embryo implantation and that high TNF alpha concentrations seem to be predictive of implantation failure. However, we could not find the elevation of TNFα in the EM of human embryos (D3). In this study, the potential of interleukin 8 (IL-8) in the EM of HPE have been determined, and the relationship of the IL-8 with embryo quality and the outcome of clinical pregnancy has been investigated. The EM from HPE (D3) of IVF/ICSI patients was collected and luminex high-throughput protein analysis was used to determine the contents of cytokines in the samples. The results showed that in patients with media from transferred embryos being tested positive for IL-8 (IL-8 positive group), the pregnancy rate, implantation rate and number of live births per in vitro fertilization (IVF) or intra-cytoplasmic sperm injection patient (N LBPP) were higher than that in patients with media being tested negative for IL-8 (IL-8 negative group), and the positive predict value of the IL-8 for predicting the chance of pregnancy was 56.86%. Compared with the IL-8 negative group, a higher pregnancy rate was observed in the IL-8 positive group when the patients received equal quality embryos. Thus, in the EM from HPE, IL-8 may be an independent predictor for pre-transfer assessment of the embryo development potential in IVF patients.

Speaker Biography

Guanyou Huang has his expertise in reproductive immunology and embryo development potential improving the progress of assisted reproductive technique. He raises the hypothesis for prediction of embryo developmental potential and pregnancy based on immune characteristics. He intends to establish a system to assess embryo quality, namely, establish a new and effective system to assess embryo quality on the basis of secretory and immune function of corresponding embryo.

e: guanyouhuang@hotmail.com

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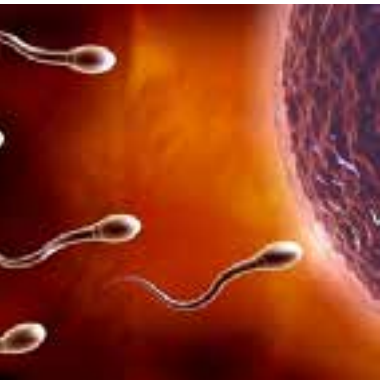

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Non-invasive mass spectrometric viability assessment of *in vitro* fertilized embryos using the alpha-1 chain of human haptoglobin

Gergely Montskó, Krisztina Gödöny, Ákos Várnagy, József Bódis and **Gábor L Kovács**
University of Pécs, Hungary

Background: Viability assessment prior embryo transfer is a crucial question since the success rate of IVF experiments is below expectations (approx. 30%). The practice of multiple embryo transfer to overcome this limitation of IVF is sometimes accompanied by multiple gestation adding several new risk factors. The leading consensus is that the best possible option is the practice of single embryo transfer. The adoption of this policy however requires the best tools to predict the embryo's implantation potential during the first couple of days of development. In parallel with the use of the routinely used morphology based viability assessment assay known as the Istanbul Consensus Scoring System (ICCS), huge effort is made worldwide to find new markers of embryo viability, preferably in a non-invasive way due to ethical issues. The search for markers of embryo viability in the embryo culture medium seems to be an ideal approach. The aim of our work was to find any biomarker present in the embryo culture medium using mass spectrometry, which would qualitatively or quantitatively differ in the samples of viable and non-viable embryos, and help predicting implantation potential.

Methods: Spent embryo culture medium samples (n=201) were measured in a series of retrospective, blind experiments, all were suitable for transferation according to the ICCS. No sample preparation was made, 15 µl of sample was directly injected into the instrument after the addition of internal standard solution. A Dionex Ultimate 3000 (Dionex Corp., USA) analytical HPLC equipped with an autosampler and a column thermostat set at 30ºC was used. Separation was carried out on a Kinetex C18 2.6 µm, 2.1 x 100 mm analytical column (Phenomenex, USA) with a multi-step gradient elution at a flow rate of 200 µL/min. The mass spectrometer coupled was a Bruker micrOTOF accurate mass instrument (Bruker Daltonik, Germany) equipped with an electrospray ionization source (ESI) operated in the positive ion mode.

Results: A protein marker was found which significantly ($p<0.001$) differed in quantity between the samples of embryos which did (clinical pregnancy), or did not (no pregnancy) implant. Respective lots of unconditioned culture media were used as controls. Deconvolution of the obtained mass spectra revealed that this protein has a molecular mass of 9186.4 Da. The protein was identified using tandem mass spectrometry as the alpha-1 chain (HptA1) of the human haptoglobin (Hpt) molecule. It was observed that Hpt was present as a contaminant in the purified human serum albumin used to supplement the culture medium therefore it is not secreted by the embryo. A significant correlation ($p<0.001$) was found when comparing the clinical outcome (clinical pregnancy or no pregnancy) and the amount of HptA1. The positive predictive value (PPV) of the biochemical analysis was 51.2% while the negative predictive value (NPV) was 100%. On the current material the ICCS assay performed a PPV of 31.3%.

Discussion: The increased amount of HptA1 in the media of non-viable embryos compared viable embryos is caused by the increased reduction of the intramolecular disulphide bonds within Hpt. This reaction (leading to HptA1 liberation) is catalyzed in a higher extent by embryos with failed pregnancy outcome. The biochemical evaluation can select embryos having good morphological aspects but low implantation potential due to visually (microscopically) unnoticeable reasons. The increased PPV observed (51.2% vs. 31.3%) is due to the fact that the mass spectrometric analysis theoretically decreased the number of false positive cases of ICCS by 40% (n=78). Since the assay has an NPV of 100% these 40% were all true negative cases. The results suggest a possible contra selection tool, screening the embryos with good morphological aspects, but no implantation potential.

e: montsko.gergely@pte.hu

Accepted Abstracts

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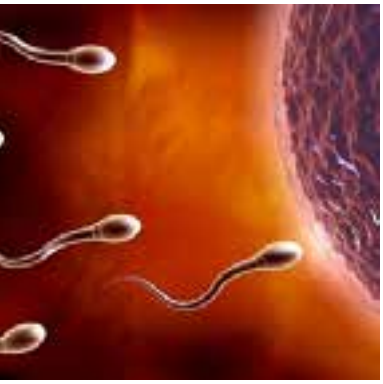

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Embryology of arrhytmogenic right ventricular dysplasia (ARVD)

Guy Hugues Fontaine
University Pierre et Marie Curie, Paris, France

A case of arrhytmogenic right ventricular dysplasia (ARVD) was discovered during routine echosonography at 24 weeks of gestation. The four-chamber view showed a large aneurysmatic area extending from below the tricuspid valve to the insertion of the moderator band; the affected wall appeared thin and akinetic, with no flow at color Doppler investigation, and no evidence of cardiovascular failure. The size of the aneurysmatic area was unchanged at subsequent controls (25 and 26 weeks of gestation). Arrhythmias could be ventricular or atrial since the involvement of the atrium is frequent. The pregnancy ended in spontaneous abortion at 27 weeks. The histopathologic examination of the heart showed the presence of adipocytes interspersed with myocardial fibers, confirming the diagnosis of ARVD. The zone of highest amount of adipocytes was located in the mediomural layers confirming where the disease starts in the embryo. This is logical because anomalies in desmosomes is the most frequent genetic factor. As the right ventricle is made of two perpendicular layers it is possible to suspect that during embryogenesis a shearing effect is taking place between the two layers especially because qtg that time the right ventricle was systemically generating strong biomechanical forces. Subsequently the sub epicardial layers were affected more severely than the subendocardial layers. When the subepicardial layers were almost completely distroyed the disease seemed to start from the epicardium towards the endocardium. However, some remnants could be visible in most of the cases if this is observed carefully. This case remains unique in the literature.

e: guy.fontaine2@numericable.fr

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Autologous platelet-rich plasma (PRP) infusions and biomarkers of ovarian and endometrial rejuvenation and aging mitigation

Aaron Traywick[1] and **E Scott Sills**[2]
[1]Inovium Rejuvenation, USA
[2]Center for Advanced Genetics, USA

The purpose of two separate clinical trials was to study and evaluate the beneficial effects of infusions of autologous platelet-rich plasma on fertility, the aging process, and general and reproductive health using blood and hormone biomarkers in peri-menopausal and menopausal women seeking fertility. Data generated as a result of the trial will be used to support additional organ-specific and disease-specific PRP-based treatments, particularly in brain and musculoskeletal tissue and stem cell rejuvenation treatments in women, as part of an ongoing research collaborative with researchers at UC-Berkeley. The most prominent data as generated from nearly 100 women participating in the 2017 trials will be presented.

e: aaron@ascendancebiomed.com

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Assessment of reactive oxygen species production in semen by the nitroblue tetrazolium reduction assay (NBT)

Afifa Sellami[1, 2]

[1]Medical School of Sfax, Tunisia

[2]Hedi Chaker Academic Hospital, Tunisia

Introduction: The presence of high reactive oxygen species (ROS) levels in semen is a major factor involved in the decline of male fertility by impairing sperm motility and DNA integrity. In semen, ROS are mainly produced by leucocytes and abnormal sperm. We aimed to assess in situ the ROS production in spermatozoa and leucocytes in the ejaculate of infertile men using photometric nitroblue tetrazolium (NBT) reduction assay.

Material & Methods: 38 semen samples from infertile patients were investigated by semen analysis and stained for ROS production with nitro blue tetrazolium. The measurement of spermatozoa and leucocytes ROS generation was evaluated via production of coloured formazan.

Results: The mean value of positive staining NBT (NBT +) sperm was 9.4% (2-35%). NBT (+) sperm rates were significantly higher in the teratospermic group in comparison with the normal morphology sperm group. We noted positive and significant correlations between the levels of (NBT +) staining sperm and some sperm morphological abnormalities: cytoplasmic droplets, flagellar angulation and coiled tails. Moreover, leucospermia was found in 28.9% of our samples and we noted a significant negative correlation between (NBT +) staining leucocytes in semen and typical morphology spermatozoa rates.

Conclusion: The NBT test is an inexpensive and easy-to-perform assay that can be routinely applied to identify sperm oxidative stress in infertile men. This assay can be used for the evaluation of seminal leucocytes activation state. It can also have significant clinical utility by the exploration of some unexplained infertility and *in vitro* fertilization failures in infertile couples.

e: sellamiafifa@yahoo.fr

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Male infertility as a circumstance of discovery of genitourinary tuberculosis

Afifa Sellami[1], Salima Daoud[1], Nozha Chakroun[1], Dorra Lahieni[2], Mounir Ben Jemaa[2] and **Tarek Rebai[1]**
[1]Medical School of Sfax, Tunisia
[2]Hedi Chaker Academic Hospital, Tunisia

Introduction: The paucisymptomatic presentation and the insidious evolution of genitourinary tuberculosis in humans induce diagnostic difficulties especially in the context of male infertility. We report two cases of urogenital tuberculosis discovered during the exploration of infertile men.

Observations & Methods: Observation 1- 40-year-old patient living in urban area was explored for primary four years infertility. Repeated spermograms were carried out according to WHO method showed severe necrospermia (vitality10-15%) with low motility (total motility<5%) and normal sperm count. Moreover, the patient presented urogenital symptomatology with dysuria, pollakiuria and post-ejaculatory pains. Spermocultures were negative. Ultrasound exploration showed a right epididymal cyst and a left epididymal nodule. Urogenital tuberculosis was suspected in a latter manifestation of fever and detection of cervical adenopathy. Observation 2- 37-year-old patient living in rural area and was explored for two years primary infertility. He reported urogenital symptomatology with painful urination, severe dysuria and recurrent hemospermia. Spermogram showed necrospermia (38% vitality), asthenospermia (total mobility 25%), leucospermia (1.6 $\times 10^6$ leucocytes/ml) and hemospermia. The evolution was marked by the persistence of hemospermia and the alteration of sperm parameters despite the prescription of antibiotherapy leading to suspicion of tuberculosis.

Results & Conclusion: The diagnosis of urogenital tuberculosis was confirmed in both patients by identification of *Mycobacterium tuberculosis* respectively at the lymph node biopsy and the urine culture. The diagnosis of urogenital tuberculosis remains difficult outside an evocative context. Its serious risk related to male infertility is linked to possibility of caseous melting of the testis and to the often irreversible damage of epididymis.

e: sellamiafifa@yahoo.fr

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Effect of different cryoprotectant agents on spermatogenesis efficiency in cryopreserved and grafted neonatal mouse testicular tissue

Yildiz C, Mullen B, Jarvi K, McKerlie C and **Lo KC**
Cengiz Yildiz
University of Mustafa Kemal, Turkey

Restoration of male fertility associated with use of the cryopreserved testicular tissue would be a significant advance in human and animal assisted reproductive technology. The purpose of this study was to test the effects of four different cryoprotectant agents (CPA) on spermatogenesis and steroidogenesis in cryopreserved and allotransplanted neonatal mouse testicular tissue. Hank's balanced salt solution (HBSS) with 5% fetal bovine serum including either 0.7 M dimethyl sulfoxide (DMSO), 0.7 M propylene glycol (PrOH), 0.7 M ethylene glycol (EG), or glycerol was used as the cryoprotectant solution. Donor testes were collected and dissected from neonatal pups of CD-1 mice (one day old). Freezing and seeding of the testicular whole tissues was performed using an automated controlled-rate freezer. Four fresh (non-frozen) or frozen-thawed pieces of testes were subcutaneously grafted onto the hind flank of each castrated male NCr nude recipient mouse and harvested after 3 months. Fresh neonatal testes grafts recovered from transplant sites had the most advanced rate of spermatogenesis with elongated spermatid and spermatozoa in 46.6% of seminiferous tubules and had higher levels of serum testosterone compared to all other frozen-thawed-graft groups ($p<0.05$). Fresh grafts and frozen-thawed grafts in the DMSO group had the highest rate of tissue survival compared to PrOH, EG, and glycerol after harvesting ($p>0.05$). The most effective CPA for the freezing and thawing of neonatal mouse testes was DMSO in comparison with EG ($p<0.05$) in both pre-grafted and post-grafted tissues based on histopathological evaluation. Likewise, the highest level of serum testosterone was obtained from the DMSO CPA group compared to all other cryoprotectants evaluated ($p<0.05$). The typical damage observed in the frozen-thawed grafts included disruption of the interstitial stroma, intercellular connection ruptures, and detachment of spermatogonia from the basement membrane. These findings indicate that neonatal mouse testes were most effectively preserved when frozen with HBSS medium with DMSO and that the type of CPA is a significant factor to obtain the most advanced stages of spermatogenesis and steroidogenesis after cryopreservation, thawing, and transplantation of neonatal mouse testes.

e: cyildiz@mku.edu.tr

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Epigenetic mechanisms in the differentiation of neural stem cells in the developing brain

Isa Abdi Rad
Urmia University of Medical Sciences, Iran

The process of brain development encompasses different stages of neurogenesis, migration, differentiation, apoptosis, arborization, synaptogenesis, synaptic sculpting and myelination. Neural stem cells (NSCs) that transform from neuroepithelial cells have a multipotential ability to give rise to neurons and glial cells, that is, astrocytes and oligodendrocytes. Differentiation of NSCs that line the neural tube is tightly regulated spatiotemporally by many genetic factors and epigenetic modifications, which can interact with transcription factors and environmental factors. Epigenetics modifications influence genes activation and silencing at different steps of NSCs differentiation through DNA methylation, histone modification, and non-coding RNAs expression without changes in the DNA sequence. Neuronal differentiation in mid-gestation, which precedes glial differentiation, is induced by epigenetic mechanisms through regulation of neurogenic basic helix-loop-helix (bHLH) transcription factors such as Ngn1, Ngn2, and Mash1. Thereafter, at late gestation, DNA methylation in astrocyte-specific promoter results in glial cells differentiation. So, DNA methylation is one of the essential epigenetic factors in differentiation of NSCs during development. DNA methylation is carried out through cytosine methylation of genomic DNA at CpG dinucleotides, which directly interferes with the binding of transcription factors to the target sequences by a family of DNA methyltransferases (DNMTs). The DNMTs family is essential for embryogenesis as their functions are necessary for maintenance of methylation patterns during DNA replication (DNMT1), and for *de novo* methylation (DNMT3a and DNMT3b). Histone modification is very complex epigenetic mechanism compared with DNA methylation. H3 and H4 core histones are modified by methylation, acetylation of lysine residue, phosphorylation, ubiquitylation, glycosylation, biotinylation, carbonylation and ADP-ribosylation. Non-coding RNAs such as microRNAs and long non-coding RNA also play roles in gene expression by transcriptional and post-transcriptional regulation, so affects the sequential differentiation of NSCs during brain development. In this seminar, the author would like to discuss epigenetic mechanisms such as DNA methylation, histone modification, and non-coding RNAs expression that are involved in the differentiation of neural stem cells in the developing brain.

e: isaabdirad@yahoo.com

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Isolated unilateral symptomatic pleural effusion-an atypical presentation of ovarian hyper stimulation syndrome-a case report

Sangeeta Sinha and **Rajesh Sinha**
Srijjan Bhilai Center, India

Abstract: An uncommon presentation of ovarian hyper stimulation syndrome is isolated pleural effusion reporting a case of late onset of ovarian hyper stimulation with unilateral pleural effusion and respiratory distress as a sole manifestation after embryo transfer.

Introduction: OHSS is one of the most grave and iatrogenic complication of controlled ovarian stimulation, clinical manifestation varying from mild to severe, it accounts for 33% of stimulated cycle. Pulmonary manifestation accounts for 7.2% of severe OHSS. But the Isolated finding of pleural effusion without ascites as the main presenting symptom of OHSS is not frequently reported and its pathogenesis is also unknown or remains a mystery. Awareness about the disease can lead to early pickup of such cases and better management. The article reports an unusual case of isolated pleural effusion after controlled ovarian stimulation after IVF and review of literature.

Case History: A 28 years old female married for eight years, no issue bilateral block on laparoscopy there was mild endometriosis no spill. So was taken for IVF. Patient had no past history of COPD, asthma, TB, no family history of chronic illness. Pt was down regulated with oral pills and lupride, D2 FSH-3.77, LH-2.93, E2-29.9. She was stimulated with 150 IU of recombinant for five days and then HMG 150 IU for another five days. At the time of HCG injection E2- 4440, and 8oocyte were retrieved. Pt was comfortable and discharged. D3 transfer was done three grades A embryo was transferred, pt discharged home comfortably. Seven days post ET patient had complain of right side chest tightness, shortness of breath, especially while lying on right side (orthopnea) dry cough. On examination her abdomen was soft no evidence of ascitis, pulse rate was 102/min, blood pressure 100/70 mm of Hg, O2 saturation was 92%, and diminished air entry on right side. Her WBC count was 15,000cells/Ul, her renal function test and liver function test was normal. Chest x-ray showed moderate to severe pleural effusion right side. Ultrasound showed no evidence of ascitis, slightly enlarged ovary. Patient was managed conservatively with a multidisciplinary approach and intensive care monitoring. She was placed in propped-up position along with antibiotic , antacid, nebulisation and chest physiotherapy looking over the amount of fluid and patient distress pleural tapping was done and 600 ml of straw colour fluid was aspirated, send for cytology and culture which was sterile and was exudates. Due to distress retapping was done after two days , patient recovered in another two days, unfortunately her beta HCG did not came to be positive, but she was discharged is good condition.

Discussion: OHSS usually result from stimulation of ovaries by Gonadotropin with the initial onset following the administration of exogenous HCG. In my case patient was young with low BMI presented six days after transfer (late onset) and was managed conservatively.

Conclusion: It demonstrates that pleural effusion may be the only manifestation of OHSS and implies a careful management of patients with pulmonary complaints after treatment with exogenous gonadotropin, so the awareness about this isolated extra-ovarian problem is very important for early and better management.

e: sangeetasinha1988@yahoo.co.in