

# IVF WORLDWIDE

ONLINE CONGRESS



## 3<sup>RD</sup> IVF-WORLDWIDE ONLINE CONGRESS IN REPRODUCTIVE MEDICINE

April 2-3, 2021

*E-Abstract Book*





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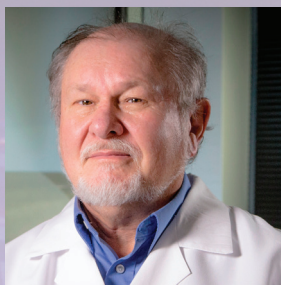


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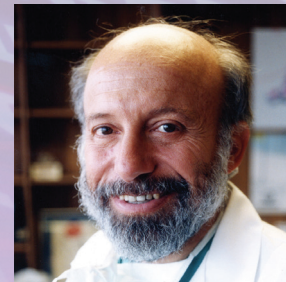
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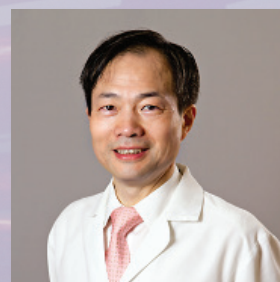
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### 3D VASCULARITY: A NOVEL PREDICTOR OF FET SUCCESS?

Vohra, Rashmi<sup>1</sup>

<sup>1</sup>Sriher, Chennai, India

#### ABSTRACT

**Introduction:** Endometrial blood flow reflects uterine receptivity. A study was done to look for a better predictor of endometrial receptivity with novel 3D technique.

**Methods:** Prospective cohort study was done on 203 women undergoing FET cycles in a tertiary care centre for a period of two years (November 2018 to November 2020) to see correlation of endometrial and subendometrial blood flow assessed by 3D power Doppler. Endometrial and subendometrial volume and their vascularisation index (VI), flow index (FI) and vascularisation flow index (VFI) were obtained and compared between pregnant and non-pregnant group. Primary outcome was clinical pregnancy and ongoing pregnancy rates.

**Results:** Out of 203 patients, 121 patients achieved pregnancy with pregnancy rate of 59.6%. The endometrial and subendometrial volume was comparable between two groups. Endometrial VI ( $3.3 \pm 1.2$  vs  $2.8 \pm 0.6$ ;  $p < 0.01$ ), FI ( $24.9 \pm 3.0$  vs  $23.1 \pm 2.7$ ;  $p < 0.01$ ) and VFI ( $1.4 \pm 0.5$  vs  $1.1 \pm 0.3$ ;  $p < 0.01$ ) were significantly higher in pregnant as compared to non-pregnant group. There was a significant difference in subendometrial VI ( $5.2 \pm 1.5$  vs  $4.4 \pm 1.9$ ;  $p < 0.01$ ), FI ( $26.0 \pm 3.5$  vs  $25.0 \pm 3.7$ ;  $p = 0.04$ ) and VFI ( $2.7 \pm 1.1$  vs  $2.2 \pm 0.6$ ;  $p < 0.01$ ) between two groups. ROC curves were plotted for these parameters and cut off values were calculated. At cut off value of endometrial VFI of 1.18, gave sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of 71.9%, 75.6%, 81.3%, 64.5% and 73.4%, respectively while at cut off value of subendometrial VFI of 2.35, gave sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of 66.9%, 63.4%, 72.9%, 56.5% and 65.5%, respectively. Cut off value of endometrial VFI of 1.18 and subendometrial VFI of 2.35, gave ongoing pregnancy rates of 67.3% and 59.5%, respectively.

**Conclusions:** Endometrial and subendometrial vascularity by 3D power Doppler can be a useful novel parameter in predicting pregnancy in FET cycles.

#### Conflict of Interest

Rashmi Vohra N/A, N Sanjeeva Reddy N/A, Radha V N/A, Monna Pandurangi N/A, N Siddhartha N/A

## 5-METHYLTETRAHYDROFOLATE SUPPLEMENTATION ENHANCE BLASTOCYST RATE AND DNA METHYLATION LEVEL IN BUFFALO

Ansari, Shama

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<sup>1</sup>ICAR-National Dairy Research Institute (NDRI), <sup>2</sup>ICAR-NDRI

### ABSTRACT

5-methyltetrahydrofolate (5-MTHF) is the predominant form of dietary folate and play a key role in one-carbon metabolism through Folate-Methylation cycle which is essential for amino acid metabolism, biosynthesis of DNA and RNA, and formation of Sadenosyl-methionine (SAM) which is the universal methyl donor for DNA, histones, proteins and lipids. Folates have an important role in prevention of neural tube defects (NTD) and aiding rapid cell division and growth of fetus. However, till date, Folate-Methylation cycle and effect of *in vitro* 5-MTHF supplementation in embryonic development of buffalo have not been studied. The present study was focussed to study the transcript expression key enzymes in the linked Folate-Methylation cycle in pre-implantation embryos. Further, to analyse the effect of 5-MTHF supplementation on blastocyst production rate and on global DNA methylation level. Using RT-PCR, we examined the gene expression pattern of the Folate-Methylation pathway enzymes in pre-implantation embryos. The *in vitro* blastocyst production was observed by giving different conc. of 5MTHF supplementation in IVC medium and also DNA methylation was detected by immunofluorescence analysis of blastocysts. Transcripts for all the enzymes of Folate-Methionine cycle (SHMT, MTHFR, MTR, MTRR, MAT1A, MAT2B, GNMT, AHCY, CBS, DNMTs, DHFR) and folate transporters (FOLR1, FOLR2, SLC19A1) were expressed in ovarian tissue. But there is the absence of some transcripts in the oocyte, and in different stages of embryos. Immunofluorescence analysis revealed FOLR2, SLC19A1 protein expression on the plasma membrane and/or cytoplasm while FOLR1 in the nucleus of pre-implantation blastocyst. The result of the present study shows that the blastocyst rate was significantly higher at 50  $\mu$ M 5-MTHF ( $26.94 \pm 2.0$ ) compared with control ( $18.80 \pm 1.0$ ). Also, 5-MTHF increases the global DNA methylation levels in blastocysts. Thus, this study advocates the necessity for examination the result of folate supplementation throughout *in vitro* embryo production for improving the quality of the transferable blastocysts and subsequently live calf births in buffalo.

### Conflict of Interest

Shama Ansari N/A, Sikander Saini N/A, Satish Kumar N/A, Dhruva Malakar N/A

# ADOLESCENT TRANSGENDER FEMALES PRESENT IMPAIRED SEMEN QUALITY THAT IS SUITABLE FOR INTRACYTOPLASMIC SPERM INJECTION EVEN BEFORE INITIATING GENDER-AFFIRMING HORMONE TREATMENT

Amir, Hadar<sup>1</sup>; Perl, Liat<sup>2</sup>; Barda, Shimi<sup>1</sup>; Lantsberg, Daniel<sup>1</sup>; Segev Becker, Anat<sup>2</sup>; Israeli, Galit Israeli<sup>2</sup>; Azem, Foad Azem<sup>1</sup>; Oren, Asaf<sup>2</sup>

<sup>1</sup>Racine IVF Unit, Fertility Institute, Lis Maternity Hospital, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel, affiliated to the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel., <sup>2</sup>Pediatric Endocrinology and Diabetes Unit, Dana-Dwek Children's Hospital, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel, affiliated to the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

## ABSTRACT

**Introduction:** The age of individuals seeking treatment for gender affirmation has fallen sharply in recent years and many of them are adolescents. Recent data exhibit impaired semen quality among adult transgender women who preserve fertility before exposure to gender-affirming hormone (GAH) therapy, but little is known about pubertal transgender female adolescents. Our aim was to determine the semen quality and cryopreservation outcomes among adolescent transgender females at the time of fertility preservation (FP) before initiating GAH treatment.

**Methods:** This retrospective cohort study included 26 adolescent transgender females who underwent FP in our Fertility Institute between 06/2013-10/2020. Pre-freezing semen parameters were compared to WHO 2010 reference values. Post-thaw semen parameters were used to determine the adequate assisted reproductive technology (ART). A multivariate linear regression analysis was performed to assess the impact of medical and lifestyle factors on semen quality.

**Results:** The mean age at which adolescent transgender females underwent FP was 16.2±1.38 years. The median values of all semen parameters in our study group were significantly lower compared to the WHO data, including volume (1.46 ml vs 3.2 ml, respectively,  $P = 0.001$ ), sperm concentration ( $28 \times 10^6/\text{ml}$  vs  $64 \times 10^6/\text{ml}$ ,  $P < 0.001$ ), total sperm number ( $28.2 \times 10^6$  vs  $196 \times 10^6$ ,  $P < 0.001$ ), total motility (51.6% vs 62%,  $P < 0.001$ ), and normal morphology (2% vs 14%,  $P < 0.001$ ). The frequency of semen abnormalities was teratozoospermia 72%, hypospermia 52%, oligozoospermia 28% and azoospermia 4%. The median post-thaw total motile count was  $0.17 \times 10^6/\text{vial}$ , and the quality was adequate only for ICSI in 87.7% of the thawed semen samples. No correlation was found between selected medical and lifestyle factors and poor semen parameters.

**Conclusions:** Semen quality is strongly reduced among adolescent transgender females before hormone therapy and their stored sperm samples are suitable for intracytoplasmic sperm injection (ICSI) rather than conventional IVF/intrauterine insemination (IUI).

## Conflict of Interest

Hadar Amir N/A, Liat Perl N/A, Shimi Barda N/A, Daniel Lantsberg N/A, Anat Segev Becker N/A, Galit Israeli N/A, Foad Azem N/A, Asaf Oren N/A

## ALUMINIUM ADMINISTRATION IS ASSOCIATED WITH BIOCHEMICAL PARAMETERS AND HISTOPATHOLOGICAL CHANGES IN RAT BRAIN: IMPACT OF A-LIPOIC ACID (A A-L)

Demmouche, Abbassia<sup>1</sup>

<sup>1</sup>Biotoxicology Laboratory. University Djillali Liabes. Faculty of Science and Life. Department of Biology

### ABSTRACT

**Objective:**  $\alpha$ -Lipoic acid (A  $\alpha$ -L) is a pleiotropic compound with a potential pharmaceutical therapeutic value against a series of physiopathological insults. The purpose of this study was to evaluate the protective role of  $\alpha$ -lipoic acid against AlCl<sub>3</sub> -induced toxic effects in Albinos Wistar rats.

**Methods:** The experiment was carried out on 50 female rats divided into five groups of 8 rats each: control group (A), group 2 treated with (AlCl<sub>3</sub>) at a rate of (100 mg/kg body weight), group 3 treated with lipoic alpha acid (A  $\alpha$ -L); put 4 together (ALCL3 In  $\alpha$ -L) treated by the chloride of aluminium and the acid alpha lipoïque (100 mg / kg of bodily weight) at the same time and put 5 together (In  $\alpha$ -L-ALCL3) treated by the acid alpha lipoïque after intoxication by the aluminium chloride.

**Results:** Our results showed that a significant increase ( $p = 0.001$ ) in body weight in the aluminum chloride-intoxicated groups compared to the control. With regard to histological cuts, the results clearly showed serious alterations (the appearance of serious cell lesions, infiltration of inflammatory outbreaks and tissue degeneration in liver parenchyma; with the appearance of fibro-vascularized partitions between the progestogen bodies in the ovaries. Exposure to AlCl<sub>3</sub> has caused histopathological damage to the brain that occurs through cell destruction marked by vascular congestions of necrotic plaque Spongy vaccination and the presence of edema. In contrast, in rats treated with alpha lipoic acid, a cell richness of type (glial cells) and nissl body was noted in our study.

**Conclusion:** It can be said and probably that A  $\alpha$ -L, as a dietary supplement, has a potential role in cognitive functions and the improvement of the cholinergic system, making it an enviable therapeutic candidate for the treatment of neurodegenerative disorders

**Key words:** Aluminium, alpha lipoic acid, wistar rats; histology; anti-oxidizer; brain

### Conflict of Interest

Demmouche Abbassia\*, Bekhadda H., Menadi AE, Bouazza S.

## **AN ASSOCIATION BETWEEN MATERNAL LEAD LEVELS AND BIRTH WEIGHT OF THE BABIES IN WEST ALGERIAN POPULATION (SIDI BEL ABBES REGION)**

Demmouche, Abbassia<sup>1</sup>

<sup>1</sup>Biotoxicology Laboratory. University Djillali Liabes. Faculty of Science and Life. Department of Biology

### **ABSTRACT**

**Background:** Several epidemiological studies have investigated high lead (Pb) exposure and pregnancy outcomes, but few studies have investigated the association of low lead exposure and low birth weight (LBW). The aims of this study were to estimate the maternal blood lead levels (BLL), to identify determinants for BLL among parturient woman and to evaluate the association of maternal BLL and LBW.

**Methods:** From July 2017 to February 2018, we carried out a case control study in the gynecology and obstetrics hospital of Sidi Bel Abbes, Algeria. Lead concentrations in maternal blood samples collected at delivery were measured in 29 mother who delivered term LBW cases group and 29 mother who give birth to a term normal weight baby matched controls. Blood lead levels were analyzed by inductively coupled plasma mass spectrometry.

**Results:** Mean maternal BLL were higher among normal groups than in mothers of LBW, but this difference was not significant ( $23,076 \pm 16,120$ , versus  $18,086 \pm 6,641$  ug/l  $p=0.247$ ). Our results indicate that the mean lead level was not higher in LBW neonates, and the whole blood lead was not related to the birth weight. In addition, there was interaction of daily kohl use and maternal BLL.

**Conclusion:** This study suggests that maternal BLL was not significantly associated with LBW. Mothers with daily use of kohl during pregnancy were more likely to have elevated blood lead concentrations.

**Key words:** case-control study, low birth weight, Algeria, lead, kohl.

### **Conflict of Interest**

Demmouche Abbassia, Moussaoui Faiza



# ANTIOXIDANT SUPPLEMENTATION DOES NOT AFFECT THE NUMBER OF OOCYTES OR FERTILIZATION RATE BUT POTENTIALLY IMPROVES BLASTOCYST GRADING AND PREGNANCY RATE.

Sinha, Priyanka<sup>1</sup>; Rizk, Botros<sup>2</sup>; Mulekar, Madhuri<sup>3</sup>

<sup>1</sup>Elite IVF, Houston, Texas, <sup>2</sup>Elite IVF center, Houston, Texas, USA, <sup>3</sup>University of South Alabama

## ABSTRACT

**Introduction:** The role of oxidative stress in female infertility has been evaluated over the last decade. Recent studies suggested an increase in clinical pregnancy rates after supplementation of the culture media with antioxidants [1]. We designed this pilot study to investigate the potential of antioxidants to improve pregnancy rates in assisted reproductive technology (ART) [2].

**Materials and Methods:** This was a prospective study of ART patients supplemented with oral Antioxidants, FH PRO for ninety days prior to ART, compared with a historical control of patients who completed their cycles during the prior 18 months. During the period from October 2018 to July 2020, seventy-four patients signed the consents of which 48 enrolled for the study while the remaining dropped out of the study. Patients in the study group were compared to a historical control of 56 patients that did not receive any antioxidants.

**Results:** The clinical pregnancy rate for the study group supplemented with the antioxidants was compared with that for the control group. The clinical pregnancy rates were 66.67% in the Antioxidant group compared to 35.19% in the control group and significant association was observed between groups ( $p=0.0096$ ).

The fertilization rate was 46% in the study group versus 56% in the control group and there was a trend towards improvement of blastocyst formation rate (43% in the study group versus 41% in the control group). Antioxidant supplementation potentially improved blastocyst grading. No significant differences were observed in the mean MII oocytes retrieved, mean fertilization (%) and mean blastulation rate between the study and the control groups.

**Conclusions:** This pilot study suggests a significant increase in clinical pregnancy rate in ART patients supplemented with antioxidants compared to those without it. Antioxidant supplementation potentially improved blastocyst grading. There was no statistically significant difference in the fertilization rates and the blastulation rates of the two groups.

## Conflict of Interest

Botros Rizk N\A, Priyanka Sinha N\A, Madhuri S. Mulekar N\A



## CAN DAY 0 OF AN ICSI CYCLE BE INDICATIVE OF LABORATORY OUTCOME

Maziotis, Evangelos<sup>1</sup>; Sfakianoudis, Konstantinos<sup>2</sup>; Giannelou, Polina<sup>3</sup>; Grigoriadis, Sokratis<sup>3</sup>; Rapani, Anna<sup>3</sup>; Tsioulou, Petroula<sup>3</sup>; Nikolettos, Konstantinos<sup>4</sup>; Pantou, Agni<sup>2</sup>; Tiptiri-Kourpeti, Angeliki<sup>4</sup>; Koutsilieris, Michael<sup>3</sup>; Nikolettos, Nikolaos<sup>1</sup>; Pantos, Konstantinos<sup>2</sup>; Asimakopoulos, Byron<sup>1</sup>; Simopoulou, Mara<sup>3</sup>

<sup>1</sup>Democritus University of Thrace, <sup>2</sup>Genesis Athens Clinic, <sup>3</sup>National and Kapodistrian University of Athens, <sup>4</sup>Embryokosmogogenesis IVF unit

### ABSTRACT

This prospective observational study aims to delineate associations between oocyte characteristics, oocyte behavior during intracytoplasmic sperm injection (ICSI), and laboratory outcomes. Recruitment included 477 patients yielding 3452 oocytes between 2015-2018. Zona pellucida (ZP) characteristics were associated with oocyte ICSI behavior ( $p < 0.001$ ). A thin ZP was further associated with sudden oolemma breakage, whereas a thick ZP with sudden oolemma breakage and fertilization failure ( $p < 0.001$ ). Abnormal ooplasm characteristics were associated with abnormal ICSI behavior ( $p < 0.001$ ), and poor fertilization evaluation outcomes ( $p < 0.001$ ). Ooplasm granularity was associated with poor embryo quality and higher probabilities of post-ICSI oocytes and embryos discarded in any developmental stage and never selected for embryo transfer or cryopreservation ( $p < 0.001$ ). Large oocyte size was associated with high resistance during ICSI ( $p < 0.001$ ). Large polar body (PB) size was associated with difficult oolemma breakage ( $p < 0.001$ ) and its respective granularity with lack of resistance during ICSI ( $p < 0.001$ ). Large perivitelline space (PVS) was associated with either types of abnormal oolemma breakage, while PVS granularity was associated with high resistance during ICSI and difficult oolemma breakage ( $p < 0.001$ ). Both abnormal oolemma breakage, and high or lack of ICSI resistance were associated with either poor Z-Score or fertilization failure ( $p < 0.001$ ). Sudden or difficult ooplasm aspiration and high resistance during ICSI penetration were positively associated with a post-ICSI oocyte or embryo that would be discarded. Evaluation of oocyte characteristics and behavior during ICSI may provide early information regarding laboratory and cycle outcomes. Particularly, ooplasm granularity, and fragmentation of polar body, along with sudden or difficult ooplasm aspiration and high or lack of resistance during ICSI penetration may hinder the outcome of an ICSI cycle. The associations presented herein may contribute towards development of a grading system or a prediction model. Taking into account information on oocytes and ICSI behavior may effectively assist in enhancing IVF outcome rates.

### Conflict of Interest

Evangelos Maziotis N/A, Konstantinos Sfakianoudis N/A, Polina Giannelou N/A, Sokratis Grigoriadis N/A, Anna Rapani N/A, Petroula Tsioulou N/A, Konstantinos Nikolettos N/A, Agni Pantou N/A, Angeliki Tiptiri-Kourpeti N/A, Michael Koutsilieris N/A, Nikolaos Nikolettos N/A, Konstantinos Pantos N/A, Byron Asimakopoulos N/A, Mara Simopoulou N/A

## CLINICAL PREGNANCY RATE, IMPLANTATION RATE AND MISCARRIAGE RATE AFTER A FROZEN EMBRYO TRANSFER COMPARATIVELY ON WEEKDAY AND WEEKEND

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<sup>1</sup>TMC Fertility Centre, <sup>2</sup>thomson Hospital Kota Damansara

### ABSTRACT

**Introduction:** Frozen embryo transfer (FET) involves thawing the embryos and transferring into the uterus of women after a course of hormonal medication support. The day of transfer is largely determined by the quality of endometrium lining and inevitably, for some patients the day of transfer may fall on weekend. In this study, we attempt to compare the clinical pregnancy rate, implantation rate and miscarriage rate for FET procedure performed on weekday and weekend respectively.

**Methods:** A retrospective study on 194 patients who underwent IVF/ICSI (Intra Cytoplasmic Sperm Injection) and proceed to FET procedure later on in our centre from 2017-2020 were conducted. The data were collected and stratified into two groups; day of transfer on weekday (n=157) or day of transfer on weekend (n=37) based on the day of FET. Clinical pregnancy rate, implantation rate and miscarriage rate were then analyzed. The Chi-square statistical analysis was conducted with a significance level at  $P < 0.05$ . Both cleavage stage embryo transfer (day 3) and blastocyst transfer were included.

**Results:** The results showed no significant difference in clinical pregnancy rate (42.0% vs 59.5%,  $p=0.0516$ ) and miscarriage rate (30.3% vs 18.2%  $p=0.2689$ ) between the weekday and weekend embryo transfer. However, there was a significant difference in implantation rate between the two groups (28.9% vs 50.9%  $p=0.0014$ ).

**Conclusions:** Our data showed that implantation rate is significantly higher in the group of patients who had their embryo transfer over the weekend. Although there was no significant difference in clinical pregnancy rate and miscarriage rate, but there was a trend towards better clinical pregnancy rate in the weekend transfer group. We hypothesized that carefully planned embryo transfer timing according to the individual's endometrium quality may have benefited some patients. However, a larger sample size is needed to verify this as well as the actual reason behind it.

### Conflict of Interest

Su Xian Lee - N/A , May Chee Fam - N/A , Sze Tian Tee - N/A

## COMPARISON OF THE FERTILIZATION, BLASTOCYST FORMATION AND UTILIZATION RATE BETWEEN DOUBLE-DENSITY GRADIENT AND ZYMOT DEVICE FOR SPERM PREPARATION

Yong, Kai Boon<sup>1</sup>; Chew, Mee Fong<sup>2</sup>; Chen, Shi Yee<sup>2</sup>; Tee, Sze Tian<sup>2</sup>; Heong, Chee San<sup>2</sup>

<sup>1</sup>TMC Fertility @Thomson Hospital, <sup>2</sup>TMC Fertility@ Thomson Hospital

### ABSTRACT

**Introduction:** Sperm preparation is an essential step in IVF/ICSI treatment, and it directly affects the outcome of the treatment. This study aims to compare the fertilization, blastocyst formation, and utilization rate for sperm prepared using the double-density gradient centrifugation method and ZyMöt sperm separation device.

**Material and methods:** This prospective randomized controlled study involved sibling oocytes from nine couples fertilized using IVF/ICSI (insemination or intra-cytoplasmic sperm injection). Total 127 oocytes were categorized into double-density gradient group (n=63) and ZyMöt group (n=64). Double-density gradient included 80% and 40% layer gradients, and HEPES buffered medium for processing sperm. For ZyMöt group, 850µl raw sample was required to load into the ZyMöt device and 750µl of HEPES buffered medium needed to cover on the surface, incubated for 30 minutes, 37°C. The fertilization, blastocyst formation, and utilization rate were compared. Statistical analysis was performed using Fisher's exact test and Wilcoxon Signed-Rank test with a significant value p<0.05.

**Results:** The fertilization rate of sperm prepared using the ZyMöt device was significantly higher than double-density gradient preparation, 67.2% (43/64) versus 47.6% (30/63), P<0.05. Besides, there was also higher blastocyst formation rate shown in the ZyMöt group, 69.8% (30/43) compared to double-density gradient group, 53.3% (16/30). Similarly, the blastocyst utilization rate from ZyMöt group, 48.8% (21/43) was also higher compared to double-density gradient group, 40.0% (12/30). However, these were not significant. The mean number of blastocyst produced from ZyMöt group (3.3±1.3) was also significantly higher compared to double-density gradient group (1.8±1.2), P<0.05.

**Conclusions:** Our results show that sperm preparation using ZyMöt device has significantly improved the oocyte fertilization rate. Moreover, there was also higher blastocyst formation and utilization rate. This results in significantly higher number of blastocyst available for transfer and freezing. Alternatively, ZyMöt device can be served as a sperm separation method with fewer handling steps.

### Conflict of Interest

Kai Boon, Yong N/A, Mee Fong, Chew N/A, Sze Tian, Tee N/A, , Shi Yee, Chen N/A, Chee San, HeonG N/A

## DOES BODY MASS INDEX INFLUENCE PERINATAL OUTCOMES IN PREGNANCIES ACHIEVED WITH ASSISTED REPRODUCTIVE TECHNOLOGY?

Dornelles, Victoria<sup>1</sup>; Hentschke, Marta<sup>1,2</sup>; Telöken, Isadora<sup>2</sup>; Vasconcelos, Natália<sup>2</sup>; Cunegatto, Bibiana<sup>1</sup>; Trindade, Vanessa<sup>1</sup>; Petracco, Álvaro<sup>1</sup>; Padoin, Alexandre<sup>2</sup>; da Costa, Bartira<sup>2</sup>; Badalotti, Marinagela<sup>1</sup>

<sup>1</sup>Fertilitat - Reproductive Medicine Center, <sup>2</sup>Pontifical Catholic University of Rio Grande do Sul (PUCRS)

### ABSTRACT

**Introduction:** Few studies analysed the influence of Body Mass Index (BMI) on perinatal outcomes from assisted reproduction techniques (ART) pregnancies. Thus, the objective of this study was to compare perinatal outcomes between patients according to previous pregnancy BMI.

**Material and Methods:** Retrospective Cohort study. Patients undergoing ART (2013-2020) were divided into three groups according to BMI (kg/m<sup>2</sup>): Group 1, <25 (n=1270); Group 2, 25-29.9 (n=356) and Group 3, ≥30 (n=127). The perinatal outcomes were compared between groups using Generalized Estimating Equations and Qui-square test/post hoc, considering p<0.05.

**Results:** The groups were compared, and the following results were found comparing Group 1, Group 2 and Group 3, respectively: live birth rate (33.5%; 32.3%; 29.9%, p=0.668); cesarean percentage (91.4%; 95.6%; 97.3%, p=0.221); twin pregnancy rate (25.2%; 26.5%; 27%, p=0.940). Regarding to maternal outcomes, the following results were found: preeclampsia (2.9%; 6.1%; 6.3%, p=0.268); premature rupture of membranes (1.3%; 4.0%; 3.1%, p=0.235) and hypothyroidism (15.4%; 7.6%; 11.1%, p=0.449). Regarding to neonatal outcomes, the following results were found: LGA percentile (4.2%; 2.9%; 4.3%, p= 0.890); Apgar score of <7 at 5 minutes (2.4%; 1.8%; none, p= 0.616); malformations (1.4%; 1.8%; none, p= 0.725); postnatal death (1.6%; 1.8%; none, p= 0.728); premature birth (29.6%; 30.1%; 35.1%, p= 0.970) and intensive care (7.2%; 2.7%; 2.7%, p=0.166).

**Conclusions:** BMI seems not being associated with a higher risk of adverse neonatal outcomes in ART patients.

### Conflict of Interest

Victoria Dornelles N/A; Marta Ribeiro Hentschke N/A; Isadora Badalotti Telöken N/A; Natália Fontoura de Vasconcelos N/A; Bibiana Cunegatto N/A; Vanessa Devens Trindade N/A; Álvaro Petracco N/A; Alexandre Padoin N/A; Bartira E. Pinheiro da Costa N/A; Marinagela Badalotti N/A



## DONORS AND RECIPIENTS HAVE THE SAME RESULTS IN A SHARED OOCYTE DONATION PROGRAM

Colombo, Talita<sup>1</sup>; Kira, Ariane<sup>1</sup>; Trindade, Vanessa<sup>1</sup>; Hentschke, Marta<sup>1</sup>; Badalotti-Teloken, Isadora<sup>1,2</sup>; Farinati, Débora<sup>1</sup>; Sanseverino, Maria Teresa<sup>1</sup>; Petracco, Alvaro<sup>1</sup>; Badalotti, Mariangela<sup>1</sup>

<sup>1</sup>Fertilitat - Reproductive Medicine Center, <sup>2</sup>PUCRS University

### ABSTRACT

**Introduction:** the oocyte donation (OD) solved the problem for women that have ovarian insufficiency, repeated implantation failure *in vitro* fertilization (IVF) or recurrent pregnancy loss. In a shared oocyte donation program, donors and recipients divided the eggs obtained by de donors, that have the cost of their cycles received as compensation.

**Material and Methods:** Retrospective cohort analysis of 586 first fresh shared oocyte donation cycles performed from 1/2002 to 12/2017 at a private clinic in Southern Brazil. The outcomes of 290 cycles from donors and 296 cycles from recipients, resulting in 473 fresh embryo transfers, were compared. The egg division was equally made, whereas, at odd amount, the donor always had preference. The data were collected from an electronic database. Statistical analysis: Chi-square test, Fisher's exact test, Mann–Whitney U-test or Student t-test depending on the data distribution, and multivariate logistic regression, considering significative  $p < 0.05$ .

**Results:** The donors' age was  $30.6 \pm 3.3$  years old, and the recipients' age was  $43.4 \pm 4.9$  y/o ( $p < 0.000.1$ ). The mean number of oocyte inseminated was 6 (4-8) in donors and 5 (3-7) in recipients groups. Fertilization rates were  $72.0 \pm 21.4$  vs.  $74.6 \pm 24.2$  respectively ( $p < 0.001$ ), without clinical significance. The number of D3 good quality embryos, blastocysts, and embryos transferred was similar in both groups. Donor's and recipient's endometrial thickness was  $11.0 \pm 2.0$  vs.  $10.2 \pm 1.9$ , ( $p < 0.001$ ), without clinical significance. Implantation (46.2% vs. 48.5%,  $p = 0.67$ ), clinical pregnancy (41.9% vs. 37.7%,  $p = 0.39$ ), and live birth rates by transfer (34.2% vs. 29.1%,  $p = 0.23$ ) were similar between groups, as well miscarriage (16.3% vs. 13.3%), ectopic pregnancies (2.6% vs. 2.1%) and multiple births rates (25.6% vs. 27.7%).

**Conclusions:** OD is often the way donors can access IVF, and for recipients may be the only option for pregnancy. A shared oocyte donation program that offers good and comparable results is fair e worth to be stimulated.

### Conflict of Interest

Talita Colombo N/A, Ariane Kira N/A, Vanessa Trindade N/A, Marta Hentschke N/A, Isadora Badalotti-Teloken N/A, Débora Farinati N/A, Maria Teresa Sanseverino N/A, Alvaro Petracco N/A, Mariangela Badalotti N/A

## EMBRYO CLEAVAGE BEHAVIOUR IN INITIAL THREE CELL CYCLES PREDICTS DEVELOPMENTAL POTENTIAL AND PLOIDY STATUS

Chen, Shi<sup>1</sup>; Heong, Chee San<sup>1</sup>; Yong, Kai Boon<sup>1</sup>

<sup>1</sup>Thomson Hospital

### ABSTRACT

**Introduction:** To study if embryo division behaviour associated with blastocyst developmental potential, utilization rate and ploidy status.

**Materials & methods:** Retrospective review of 59 PGT-A cases at Thomson hospital. Embryo were cultured for 6 days using embryoscope. Division patterns in initial three cell cycles were registered and divided into 2 groups: a)normal b)abnormal. Abnormal division was defined as having one or combination of these cleavage irregularities: direct cleavage from 1 to 3 cells, direct cleavage from 1 to  $\geq 3$  cells, daughter cells cleavage time  $< 5h$ , reverse cleavage. On day 5/6, AA/AB/BA/BB blastocysts(BG3-6)(modified gardner grading system) were considered usable, and qualify for biopsy and freezing. Blastocysts with 25-80% aneuploidy in trophoctoderm cells were reported as mosaic. Parallel comparison of ploidy status was performed between normal and abnormal division group. Chi square and Mann-Whitney U test were used for analysis, significant value at  $p < 0.05$ .

**Results:** Of 391 fertilized zygotes, 51.15%( $n=200$ ) from the cohort showed normal cleavage, while 48.85%( $n=191$ ) had abnormal division. Mean age of normal and abnormal division group was  $36.3 \pm 4.7$  and  $36.58 \pm 4.8$  ( $p=0.35$ ), respectively. Timing of first cleavage of abnormal group was slightly longer than normal group ( $28.25 \pm 4.95$  vs  $25.98 \pm 2.9$  [ $p=0.00001$ ]). Blastulation rate of abnormal group was  $\sim 35\%$  significantly lower compared with normal group ( $60.7\%[116/191]$  vs  $95.5\%[191/200]$ ;  $p=0.00001$ ). Also, abnormal group had lower utilization rate than normal group ( $34\%[65/191]$  vs  $77\%[154/200]$ ;  $p=0.00001$ ). Parallel comparison showed that incidence of euploidy in normal group was significantly higher than abnormal group ( $42.9\%[66/154]$  vs  $26.2\%[17/65]$ ;  $p=0.02$ ). There was a trend towards higher mosaic rate in embryos with abnormal cleavage ( $24.6\%[16/65]$  vs  $14.9\%[23/154]$ ;  $p=0.13$ ). Similarly, aneuploidy rate of abnormal group ( $49.23\%[32/65]$ ) was higher than normal group ( $42.2\%[65/154]$ ) ( $p=0.42$ ).

**Conclusions:** Embryo with abnormal cleavage behaviour in first three cell cycles is associated with negative outcomes in terms of blastocysts formation and utilization rates, while embryos showed normal division is more likely to be euploid if they are able to form usable grade blastocysts (BG3-6, AA-BB). It is suggested embryo division patterns could be a useful measure in predicting blastocyst development potential and provide additional information on deciding which embryos to choose for transfer.

### Conflict of Interest

ShiYee Chen (N/A), CheeSan Heong (N/A), KaiBoon Yong (N/A),

## EVALUATION OF ART OUTCOMES BASED ON STIMULATION DOSAGES AND AMH LEVELS

Samsudin, Nurulhafizah<sup>1</sup>; Samsudin, Nurulhafizah<sup>2</sup>

<sup>1</sup>TMC Fertility & Women's Specialist Center (Malaysia), <sup>2</sup>TMC Fertility and Women's Specialist Centre

### ABSTRACT

**Background:** Ovarian aging is a challenge in Assisted Reproductive Technology (ART) because it causes gradual decrease in ovarian reserve. Assessment of ovarian reserve using anti-Mullerian hormone (AMH) could predict ovarian response to stimulation. Our study evaluates the follicle-stimulating hormone (FSH) and Human Menopausal Gonadotrophin (HMG) dosages on the outcomes of ART in low, normal and high responders.

**Methods:** 70 women were divided into three groups: (A) low responders (AMH  $\leq 5.4$  pmol/mL,  $n=17$ ), (B) normal responders (5.5 pmol/mL-24.9 pmol/mL,  $n=25$ ), and (C) high responders ( $\geq 25$  pmol/mL,  $n=28$ ). FSH and HMG dosages in controlled ovarian stimulation were determined. Retrieval, maturation, fertilization and utilization rates were measured. Data presented as Mean  $\pm$  SD. Comparisons were made using Student's T-test.

**Results:** The FSH and HMG dosages in low responders were  $257 \pm 134$  IU/day for  $10 \pm 4$  days and  $145 \pm 154$  IU/day for  $5 \pm 5$  days; normal responders were  $261 \pm 74$  IU/day for  $10 \pm 3$  days and  $79 \pm 94$  IU/day for  $5 \pm 5$  days; and high responders were  $173 \pm 75$  IU/day for  $10 \pm 4$  days and  $74 \pm 115$  IU/day for  $4 \pm 5$  days, respectively. Low responders had  $4.6 \pm 2.9$  oocytes retrieved, normal responders had  $14.4 \pm 9.4$  and high responders had  $18.7 \pm 8.4$ . The rates of retrieval, maturation, fertilization and utilization in low responders were  $73.4 \pm 30.4$ ,  $89.7 \pm 12.5$ ,  $68.9 \pm 30.3$  and  $71.2 \pm 26.4$ ; in normal responders were  $81.9 \pm 20.7$ ,  $79.9 \pm 13.7$ ,  $68.0 \pm 21.8$  and  $47.6 \pm 24.7$ ; in high responders were  $88.4 \pm 16.8$ ,  $79.1 \pm 18.6$ ,  $61.2 \pm 20.4$  and  $60.3 \pm 30.2$ , respectively.

**Conclusion:** Although low responders produce less oocytes ( $P < 0.05$ ), oocyte maturation and fertilization rates are not affected ( $p > 0.05$ ). Embryo utilization rate of low responders is higher than normal ( $p = 0.0035$ ) suggesting that they may produce good quality embryos with proper stimulation.

### Conflict of Interest

Nurulhafizah Samsudin (N/A), Tee Sze Tian (N/A)

## IMPACT OF MALE AGE IN ASSISTED REPRODUCTIVE TECHNIQUES

Mendes, Inês Azevedo de Sá<sup>1</sup>; Pereira, Isabel<sup>2</sup>; Aguiar, Ana<sup>2</sup>; Sousa, Sandra<sup>2</sup>; Nunes, Joaquim<sup>2</sup>; Lopes, Giendre<sup>3</sup>; Carvalho, Marta<sup>3</sup>; Leal, Fernanda<sup>3</sup>; Calhaz-Jorge, Carlos<sup>4</sup>

<sup>1</sup>Hospital de Cascais, <sup>2</sup>Reproductive Medicine Unit of Hospital de Santa Maria, Centro Hospitalar Universitário Lisboa Norte, <sup>3</sup>Clinical Embryologist at Hospital de Santa Maria, <sup>4</sup>Faculdade de Medicina da Universidade de Lisboa

### ABSTRACT

**Introduction:** There is a well-known negative impact of maternal age in the outcome of Assisted Reproductive Technology (ART). However, the influence of paternal age is still a challenge full of controversial studies.

The purpose of this work is to evaluate the influence of male age on clinical pregnancy, abortion and delivery rates in In Vitro Fertilization (IVF) and Intrauterine Insemination (IUI).

**Material and Methods:** Retrospective analysis of data collected prospectively, referring to IUI cycles (n = 642) since January 2014 and IVF cycles (n = 533) from January 2013 to June 2019, in a tertiary center. The exclusion criteria were male infertility, female smoking and/or uterine malformations.

The couples were divided in 4 groups based on female age (< 35; ≥35 years) and male age (<40; ≥40 years). IUI and IVF cycles were evaluated separately.

For data analysis, SPSS® Statistics and GraphPad were used. The correlation factor between male age, adjusted for female age, was assessed using binary logistic regression. The comparison between the outcomes in the subgroups was performed using the Fisher's exact test (two-tailed).

**Results:** In IUI, the clinical pregnancy, abortion and delivery rates were similar in all subgroups. In the IVF outcomes, we found a negative association of male age with the clinical pregnancy rate (46% vs 34%, p = 0.032) and with the delivery rate (35% vs 23%, p = 0.031) in the subgroup of women aged ≥35, with worse results for male age ≥40 years.

**Conclusions:** In our study, male age did not influence the outcomes of IVF and IUI when maternal age was <35 years. However, in the subpopulation of women aged ≥35 years, there appears to be a negative synergistic effect of advanced male age in IVF clinical outcomes.

### Conflict of Interest

Inês Mendes N/A, Isabel Pereira N/A, Ana Aguiar N/A, Sandra Sousa N/A, Joaquim Nunes N/A, Giendre Lopes N/A, Marta Carvalho N/A, Fernanda Leal N/A, Carlos Calhaz-Jorge N/A



## IUI RESULT AFTER RANDOM STIMULATION IN PATIENT WITH IRREGULAR CYCLE

Elmegrab, Hunida<sup>1</sup>

<sup>1</sup>Helmegrab

### ABSTRACT

**Background:** Recent research has suggested that recruitable antral follicles are continuously present in the ovaries during the menstrual cycle, and ultrasonographic studies have demonstrated that multiple cohorts or 'waves' of 2–5 mm follicles are recruited continuously during a menstrual cycle. A wave is a synchronous growth of follicles that have a similar diameter. Waves of follicle development have been documented in healthy women using ultrasonography. It has been proposed that there are two waves of follicular growth during the menstrual cycle. **Objective:** to determine how to enhance the chances of pregnancy by random stimulation protocol and IUI in patients with irregular cycle and poor responders.

**Method:** prospective study done at reproductive unit alhawari hospital, 15 women enrolled to study seeking for fertility with irregular cycle and diagnosed as poor responder before, there is no need to wait for the menstrual period and ovarian stimulation can be started at any time during the cycle, even after ovulation. With our unique stimulation approach, we can medically manage the cycle without a need for further delay.

**Conclusions:** By this approach to ovarian stimulation particularly applied I for irregular cycle and poor responders' women they could benefit from it. More details at presentation

### Conflict of Interest

Dr. Manal Shaban Elmansori, Dr. Lamia Abdelrahem Ballo Dr. Hunida .Elmegrab

## LEAD, CADMIUM AND MERCURY CONCENTRATIONS IN UMBILICAL CORD BLOOD AND PREMATURITY BIRTH IN SBA REGION (WEST OF ALGERIA)

Demmouche, Abbassia<sup>1</sup>

<sup>1</sup>Biotoxicology Laboratory. University Djillali Liabes. Faculty of Science and Life. Department of Biology

### ABSTRACT

**Objectives:** Exposure to heavy metals such as lead, cadmium and mercury during pregnancy carries a great risk to the mother as well as the fetus. The aim of this study was to measure in umbilical cord blood the concentration of lead (Pb), mercury (Hg) and cadmium (Cd), and evaluates the relationship between this levels and prematurity. The lead, cadmium and mercury levels were measured by atomic absorption.

**Methods:** Lead, cadmium and mercury were measured in umbilical cord blood samples of 70 women who delivered at “service of obstetrics and genecology” in Hospital-Center University of Sidi Bel Abbes region in Algeria between 2016 and 2017.

**Results:** The study showed obvious variations in, maternal characteristics, socioeconomic status and obstetric/ gynecological history for mother. The results revealed several factors predisposing to prematurity in addition, age of mother, Socio-economic level and History of abortion. The mean concentrations of cord blood lead, cadmium and mercury were; 18.97 µg/L, 0.26 µg/L, and 6.20 nmol/L, respectively. There was a highly significant direct correlation between cord lead concentrations and gestational age( $r=0.43$ ;  $P = 0.017$ ), and we found that gestational age and birth weight inversely correlated with cord mercury concentration ( $r=0.44$  and  $r=0.57$  respectively). No correlation was observed between cord cadmium concentrations and gestational age.

**Conclusions:** This study has shown that pregnant women in this region of the country were exposed to high levels for heavy metals which need an intervention.

**Keywords:** Lead; Cadmium; Mercury; Pregnancy; Prematurity; Algeria

### Conflict of Interest

Demmouche Abbassia; Bouhadiba Hadjer; Menadi Noredine; Mai Hicham; Bekhadda Hadjer; Bouazza Sofiane

## **LUTEAL PHASE OOCYTE RETRIEVAL AND INDICATIONS OF SUCCESSFUL PRACTICE**

Galatis, Dionysios<sup>1</sup>; Simopoulou, Mara<sup>1</sup>; Maziotis, Evangelos<sup>2</sup>; Pantou, Agni<sup>3</sup>; Giannelou, Polina<sup>1</sup>; Grigoriadis, Sokratis<sup>1</sup>; Tzonis, Panagiotis<sup>3</sup>; Griva, Theodora<sup>3</sup>; Zikopoulos, Athanasios<sup>4</sup>; Philippou, Anastasios<sup>1</sup>; Koutsilieris, Michael<sup>1</sup>; Pantos, Konstantinos<sup>3</sup>; Sfakianousis, Konstantinos<sup>3</sup>

<sup>1</sup>National and Kapodistrian university of Athens, <sup>2</sup>Democritus University of Thrace, <sup>3</sup>Genesis Athens Clinic, <sup>4</sup>Royal Cornwall Hospital

### **ABSTRACT**

The second follicular wave in the same menstrual cycle has been introduced as an encouraging means towards optimizing the context of in vitro fertilization (IVF) success rates for infertile women and especially for poor responders. A total of 1688 women diagnosed as poor responders according to Bologna criteria, undergoing natural cycle IVF between 2012-2020 including two oocyte retrievals in the same cycle were included in this retrospective study. Patients' age, BMI, number of previous failed IVF attempts, basal hormonal levels, AFC, E<sub>2</sub> evaluated on both trigger days and number of small follicles (8-13mm) were evaluated on their predictive power regarding retrieval of at least one oocyte following LuPOR, being regarded as successful LuPOR practice. A random 20% of each quantile was employed to validate the model. The remaining 80% was employed to develop the model. The predictive value was determined employing the Area Under the Curve (AUC) of the Receiver Operating Characteristics, employing Youden's index. AFC with a threshold of 3.92, E<sub>2</sub> on both FoPOR and LuPOR trigger days with threshold of 222.92pg/ml and 174.38pg/ml respectively, along with the number of small follicles during FoPOR, with a threshold of 1.93, are predictive of successful LuPOR. The AUC was 0.64, 0.65, 0.62 and 0.62 respectively. Specificity was 0.52, 0.77, 0.46 and 0.28 respectively. Sensitivity was 0.69, 0.77, 0.93 and 0.89 respectively. Accuracy was 0.68, 0.75, 0.89 and 0.86 respectively. When combining the above characteristics into a single predictive model the AUC was 0.64, specificity 0.56, sensitivity 0.65 and accuracy 0.65. The positive and negative predictive values was 94.61% and 10.46% respectively. The high positive predictive value of this model may assist clinicians in identifying poor responders who will benefit from double oocyte retrieval in the same cycle.

### **Conflict of Interest**

Dionysios Galatis N/A, Mara Simopoulou N/A, Evangelos Maziotis N/A, Agni Pantou N/A, Polina Giannelou N/A, Sokratis Grigoriadis N/A, Panagiotis Tzonis N/A, Theodora Griva N/A, Athanasios Zikopoulos N/A, Anastasios Philippou N/A, Michael Koutsilieris N/A, Konstantinos Pantos N/A and Konstantinos Sfakianoudis N/A

# **MOTILITY, VIABILITY AND APOPTOSIS IN CRYOPRESERVED HUMAN SPERM WHEN THAWED AT EITHER ROOM TEMPERATURE OR 37°C.**

Wang, Wenshuai<sup>1</sup>; Matson, Phillip<sup>1</sup>; Burton, Peter<sup>2</sup>

<sup>1</sup>Edith Cowan University, <sup>2</sup>Concept Fertility Centre

## **ABSTRACT**

Damage to sperm following cryopreservation can occur by the induction of apoptosis. Cryopreservation methods are not standardized, hence the present study investigated systematically the changes in frozen human sperm when thawed at either room temperature (RT) or 37°C.

Normozoospermic semen samples (n=7) were obtained from patients following informed consent. 0.5ml Quinn's Advantage™ Sperm Freezing Medium was added dropwise to 0.5ml semen. Semen in two 0.25ml straws were placed vertically for 30 minutes into a goblet suspended in a Dewar, plunged into liquid nitrogen, and stored for up to 90 minutes before thawing. One straw was thawed at RT for 3 minutes whilst the other straw was thawed by placing in 37°C water bath for 3 minutes. Sperm apoptosis was analysed by Annexin V and Dead Cell Reagent, using a Muse® Cell Analyser Flow Cytometer. Changes post-thaw were analysed by repeated measures ANOVA, and differences between thaws at RT and 37°C by paired t-test. Results with  $p < 0.05$  were considered significant.

Significant overall reductions were seen post-thaw in total motility ( $p < 0.00001$ ), progressive motility ( $p < 0.001$ ), and the proportion of live sperm ( $p < 0.0001$ ) whilst significant increases occurred in early ( $p < 0.00001$ ), late ( $p < 0.01$ ) and total apoptosis ( $p < 0.00005$ ). Compared to the RT thaw, the 37°C thaw resulted in significantly fewer (mean±sem) live sperm ( $56.3 \pm 5.3$  vs  $60.8 \pm 5.9$ ,  $p < 0.02$ ) and higher incidences in early ( $22.7 \pm 1.3$  vs  $19.2 \pm 1.2$ ,  $p < 0.03$ ) and total apoptosis ( $40.6 \pm 5.2$  vs  $36.2 \pm 5.8$ ,  $p < 0.02$ ).

In summary, thawing frozen semen for 3 minutes at RT results in similar total and progressive motility compared to thawing at 37°C, but more live sperm and fewer showing early and total apoptosis. Thawing at RT is therefore recommended.

Acknowledgements: Thanks to the staff at Concept Fertility for help with the processing of the semen samples.

## **Conflict of Interest**

Wenshuai Wang N/A, Phillip Matson N/A and Peter Burton N/A



## **MULTIPLE CONJOINED OOCYTES IN A PATIENT WITH POLYCYSTIC OVARY SYNDROME UNDERGOING IN VITRO FERTILIZATION**

Bermio, Gladys Anne<sup>1</sup>

<sup>1</sup>ACE Medical Center Quezon City

### **ABSTRACT**

**Background and Aim:** Conjoined oocytes are rarely found in reproductive age. Having only 28 reported cases to date, limited data exist among IVF centers on its potential significance. Theories to explain its existence are developmental accident and failure of meiotic division. Published studies indicate that ovarian stimulation in Assisted Reproductive Technology predisposes to its occurrence. Polycystic ovaries, on the other hand, give rise to follicles with different maturational states, thereby further contributing to the occurrence of conjoined oocytes. We present a case of multiple conjoined oocytes from one patient in an IVF facility.

**Methods:** Case report of a PCOS patient seen in IVF clinic for ART.

**Results:** Ovarian stimulation was carried out using the antagonist protocol. Oocyte retrieval was scheduled 36hours after GnRH agonist trigger, which resulted to 16 MII and 5 GV. Four conjoined oocytes, each containing MII and GV, were likewise noted. Intracytoplasmic sperm injection led to normal fertilization in 2conjoined oocytes, which then developed to blastocyst stage at 3BB and 3CB, while the other 2conjoined oocytes remained unfertilized. Preimplantation genetic screening on all surviving blastocysts showed euploid 3BB and aneuploid 3CB embryos arising from the conjoined oocytes apart from 2 other euploid embryos arising from uniovular oocytes. On frozen embryo transfer, GV attached to the euploid XX embryo at stage 3BB (conjoined) was removed and transferred together with a euploid XY embryo at stage 4BB, which resulted to healthy term twins.

**Conclusions:** Gonadotropin stimulation, coupled with PCOS, predisposes to the occurrence of conjoined oocytes. Although a result of developmental accident, conjoined oocytes still have the potential to develop into a genetically normal embryo, hence, into a normal pregnancy. To our knowledge, this reports the third case of conjoined oocyte that resulted to a live birth, and probably the highest number of conjoined oocytes retrieved in a single IVF cycle.

### **Conflict of Interest**

N/A

## **NGFβ ENHANCES IN-VITRO MOTILITY AND VITALITY OF HUMAN SPERMATOZOA**

Asimakopoulos, Byron<sup>1</sup>; Tiptiri-Kourpeti, Aggeliki<sup>2</sup>; Metallinou, Chryssa<sup>3</sup>

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### **ABSTRACT**

Progressive motility (PM) and vitality of spermatozoa are positively associated with fertilization and pregnancy rates in conventional in-vitro fertilization (IVF) and intrauterine insemination (IUI). Therefore, the enhancement of PM and vitality is a main goal during sperm processing. In this study, the effect of NGFβ on PM and vitality of human spermatozoa, by adding this growth factor in the culture medium during sperm processing, was investigated.

The study was conducted in the Laboratory of Physiology, Democritus University of Thrace, in the context of the research project “Study of the effects of growth factors on the motility and vitality of human spermatozoa” (MIS 5049528). The study was approved by the Ethics Committee of Democritus University.

Forty three male volunteers gave semen samples by masturbation after 2-3 days of sexual abstinence. Each sample, after basic semen analysis, was processed with density gradient centrifugation and sperm washing. The pellet was divided into 3 aliquots. An aliquot containing 1 million of progressively motile spermatozoa was incubated for one hour (37°C) in standard culture medium (control group) and two aliquots with the same number of progressively motile spermatozoa were incubated in culture medium supplemented with NGFβ at a concentration of 0,5ng/ml or 5ng/ml.

NGFβ significantly increased PM and vitality in comparison to control either at the low or the high concentration. However, this increase was not dose-dependent; although both parameters were higher with the high concentration, there was no significant difference of the increase of PM or vitality between high and low concentration of NGFβ.

The enhancement of PM and vitality of human spermatozoa by NGFβ opens new ways for the improvement of sperm processing and consequently for the improvement of the outcomes during IUI and conventional IVF. Further research is needed to determine the most effective concentration of NGFβ.

### **Conflict of Interest**

Byron Asimakopoulos N/A; Aggeliki Tiptiri-Kourpeti N/A; Chryssa Metallinou N/A

## OBESITY AND OVERWEIGHT ARE ASSOCIATED WITH LOWER PREGNANCY RATE IN IVF TREATMENT

Dornelles, Victoria<sup>1</sup>; Hentschke, Marta<sup>2,1</sup>; Telöken, Isadora<sup>2</sup>; Vasconcelos, Natália<sup>2</sup>; Cunegatto, Bibiana<sup>1</sup>; Trindade, Vanessa<sup>1</sup>; Petracco, Álvaro<sup>1</sup>; Padoin, Alexandre<sup>2</sup>; da Costa, Bartira<sup>2</sup>; Badalotti, Marinagela<sup>1</sup>

<sup>1</sup>Fertilitat - Reproductive Medicine Center, <sup>2</sup>Pontifical Catholic University of Rio Grande do Sul (PUCRS)

### ABSTRACT

**Introduction:** One-third of the world population is considered to be overweight or obese. In *in vitro* fertilization (IVF), the need for higher doses of gonadotropins for obese patients' ovarian stimulation is well established. However, it is still unclear whether the weight effect in fertility is translated into worse clinical outcomes after embryo transfer (ET). This study aimed to analyze the impact of body mass index (BMI) on laboratorial and clinical results of IVF treatment.

**Material and Methods:** Retrospective cohort study. Patients undergoing IVF (2013-2020) were included and divided into three groups according to BMI: Group 1 (BMI < 24.4 Kg/m<sup>2</sup>), Group 2 (BMI 25-29.9 Kg/m<sup>2</sup>) and Group 3 (BMI ≥30 Kg/m<sup>2</sup>). A total of 1753 IVF-ET cycles were included for assisted reproductive techniques (ART) outcomes analysis and 1869 IVF-ET + frozen embryo transfer (FET) for cumulative pregnancy analysis.

**Results:** The mean maternal age was 35.5 ± 3.6, 35.9±3.6, and 35±4.3 in groups 1, 2 and 3, respectively. A greater number of mature oocytes was observed in groups 1 and 2 (6 [6.4-7]; 6 [5.6-6.6]; 4 [4.6-6.7], p= 0.0111). The fertilization rate was similar between groups. Lower but not statistically significant implantation (28.4%; 27.5%; 23.2%, p=0.187), clinical pregnancy (40.1%; 39.7%; 32.5%, p=0.262) and live birth rate (33.5%; 32.3%; 29.9%, p=0.668) was found in group 3. The cumulative clinical pregnancy rate was 48.0%, 46.7% and 36.3% in groups 1, 2 and 3, respectively, with significant linear tendency (p=0.042).

**Conclusions:** Obesity negatively impacts the pregnancy chance in patients submitted to IVF treatment, probably because of the lower number of mature oocytes. The higher the BMI, the worse was the cumulative clinical pregnancy rate. Whenever possible, considering the patient's age, the ovarian reserve, and the time required for weight loss, a reduction in BMI should be sought before IVF to obtain better results.

### Conflict of Interest

Victoria Dornelles N/A; Marta Ribeiro Hentschke N/A; Isadora Badalotti Telöken N/A; Natália Fontoura de Vasconcelos N/A; Bibiana Cunegatto N/A; Vanessa Devens Trindade N/A; Álvaro Petracco N/A; Alexandre Padoin N/A; Bartira E. Pinheiro da Costa N/A; Marinagela Badalotti N/A

## OPTIMIZING ESTROGEN LEVEL IN WOMEN AT REPRODUCTIVE AGE PRIOR TO START CONTROLLED STIMULATION (CO) FOR ART

Elmegrab, Hunida<sup>1</sup>

<sup>1</sup>Helmegrab

### ABSTRACT

**Background:** Estrogen is the main hormone involved in female reproduction. All aspects of female reproduction, conception and pregnancy are controlled by estrogen. It is kind of the “king “of female hormones. Objective: to evaluate the estrogen level and its relation to follicle size using ultrasound folliculometry in women seeking pregnancy to prevent ineffective induction

**Method:** In this cross-sectional study that was conducted at reproductive unit alhawari hospital, 100 women in reproductive age seeking pregnancy were followed-up during 3 consecutive cycles using estrogen level in menstrual and follicular phase and correlating it to follicle size by ultrasound folliculometry

**Conclusion:** Patients striving for fertility treatment should always be followed-up with estrogen level and correlating it well to follicle size in order to optimize it before starting induction. More details at presentation

### Conflict of Interest

Dr. Fatimah Mustafa Essa, Dr. Enas Aloogly, Dr. Hunida .Helmegrab

# **PERSPECTIVE FOR THE USE OF LET-7 AND MIR-9 AS A NON-INVASIVE BIOMARKERS IN THE DIAGNOSIS AND ASSESSMENT OF THE EFFECTIVENESS OF TREATMENT OF PATIENTS WITH EXTERNAL GENITAL ENDOMETRIOSIS.**

Pokrovenko, Dasha<sup>1</sup>

<sup>1</sup>Dasha

## **ABSTRACT**

At the present stage, much has been done to understand the mechanisms of endometriosis, but still the "gold standard" of diagnosis is invasive methods, especially laparoscopy. The search for possible biomarkers for the diagnosis of endometriosis continues. Among non-invasive methods, the most promising for future study and research is the micro-RNA. In patient with endometriosis the micro-RNA profile of blood and eutopic endometrium may provide important information in confirming diagnosis.

We've conducted the study among patients with genital endometriosis, in which we aimed to find a link between the level of circulating mir-RNA let-7 and mir-9 in serum samples and stage of endometriosis by using genome-wide microarray approach in 2 groups of patients : 1- control, patients without endometriosis; 2- patients with endometriosis but without prior treatment (this group is divided into subgroups depending on the degree of spread of endometriosis assessed by laparoscopic intervention).

**Results:** We did not find a clear correlation between the severity of pain by VAS and the severity of endometriosis by ASRM, but at the IV stage of endometriosis, the average indicators were still higher than at the I respectively  $75 \pm 5\text{mm}$  and  $45 \pm 5\text{mm}$  ( p-value for let-7 0.04644; p-value mir-9 0.2985) Confidence interval for let-7 was (54.64-204.41) for control group, endometriosis group - (2.56-11.35) and for mir-9 respectively (0-15.54) and (0-13.30). So, on a given number of samples, we see a difference in indicators between the control groups and endometriosis groups, while for let-7 there is a correlation depending on the degree of prevalence of the process, but no correlation for mir-9.

## **Conflict of Interest**

Medvediev Mykhailo

## **PROINFLAMMATORY CYTOKINE IL-2 IN PELVIC PERITONEAL ADHESIONS OF VARIOUS ETIOLOGY AT REPRODUCTIVE AGE WOMEN**

Sulima, Anna<sup>1</sup>; Davydova, Aleksandra<sup>2</sup>

<sup>1</sup>Anna Sulima, <sup>2</sup>Aleksandra Davydova

### **ABSTRACT**

To study the expression of IL-2 in the tissue of the pelvic peritoneal adhesions of various origins in women of reproductive age.

It is known that IL-2 is a basic proinflammatory cytokine that influences on the differentiation and specialization of T and B lymphocytes, stimulating natural killer cells and macrophages. Data on expression of IL-2 in the pelvic peritoneal adhesions in connection with their prescription, localization and origin is absent at accessible literature.

One hundred infertile women (aged 19-49 yrs) with pelvic peritoneal adhesions, who were underwent operative laparoscopy. 38 patients with a history of chronic inflammatory diseases of pelvic organs; 32 patients with endometrial disease and 30 patients who had undergone previous surgery for pelvic and abdominal cavity took part in this study.

The material for this study was the fragments of surgical material (adhesions and their parts) n=100. The morphological and immunohistochemical study of adhesions were carried out by standard techniques using paraffin blocks, reagents of Dako and monoclonal antibodies to IL-2 (Anti-IL-2 antibody (ab6672)) of Abcam with automatic coloring Dako Cytomation.

The expression of IL-2 was extremely low and was equal to  $13 \pm 0,4$  points. Only a few positively stained cells were found in the vessels lumen or perivascular space. During the immunohistochemical study of adhesions in women with a history of inflammatory diseases of the pelvic organs, there was an low IL-2 also, i.e. it was  $19 \pm 0,5$  points. Immunohistochemical study of adhesions in patients with external genital endometriosis carried out in the first phase of the cycle was characterized by a moderate expression of IL-2 in the mesothelial cells and lymphocytic-macrophage aggregates. Expression of IL-2 was  $111 \pm 0,3$  points.

The low expression of IL-2 may indicate to the changing of peritoneum regeneration in the form of repair retardation and as a result the adhesions formation.

### **Conflict of Interest**

Anna Nikolaevna Sulima N/A, Aleksandra Aleksandrovna Davydova N/A

# RETROSPECTIVE STUDY OF CULTURING METHODS , ITS EFFECT ON BLASTULATION RATE AND ICSI OUTCOME.

Mishra, Pooja<sup>1</sup>

<sup>1</sup>Cocoon fertility centre,Pune

## ABSTRACT

**Introduction:** it is hypothesized that one reason for benefit of group embryo culture is embryo modification of their microenvironment due to secretion and/or depletion of various factors in the media. Some unknown factors derived from poor quality embryos may inhibit the surrounding embryo development.

**Methods and materials:** Retrospective study is performed on total 205 patient's gametes and these patients were divided into two groups (Group A and Group B) depending on their gamete culturing methods.

**GROUP A**—105 patient's oocytes were cultured by drop culture method i.e.- after ICSI each oocytes were shifted to each drop of 10ul from day1 to day 3 and again on day3 embryos were shifted to fresh culture(drop culture method) till day 5.

**GROUP B**—100 patient's oocytes were cultured by drop culture method i.e.- after ICSI each oocytes were shifted to each drop of 10ul from day1 to day 3 and on day3 those embryos were shifted to group culture till day 5. i.e.- group of 3-4 embryos (2-3 grade I embryos +1 grade II/grade III embryo)in each drop of 20ul. All patients selected were strictly blastocyst transfer patients. We compared the results of both group (embryo formation rate, blastocyst rate, pregnancy rate) the embryo formation rate, blastocyst rate, pregnancy rate were calculated and compared between Group A and Group B as well.

## RESULTS:

	CLEAVAGE RATE	BLASTOCYST RATE	PREGNANCY RATE
GROUP A	71.70%	43.68%	75.23%
GROUP B	70.64%	51.90%	77 %

**Conclusions:** to maintain a good results in art lab, we should culture embryos in group i.e. (2 to3 – grade I embryos+1-grade II /grade III embryos) ,so that autocrine factors from grade I embryos will help grade II /grade III embryos to become good quality blastocyst.

## Conflict of Interest

Miss. Pooja Mishra N/A

## REVIEW TRENDS OF OOCYTE CRYOPRESERVATION

Fam, May Chee<sup>1</sup>; CHEN, SHI YEE<sup>2</sup>; Tan, Yin Thien<sup>3</sup>; TEE, SZE TIAN<sup>4</sup>

<sup>1</sup>TMC Fertility Centre Ipoh, <sup>2</sup>Thomsom Hospital, Kota Damansara, <sup>3</sup>TMC Fertility Centre,

<sup>4</sup>Thomsom Hospital, petaling Jaya

### ABSTRACT

**Introduction:** The quality of the cryopreserved egg is inversely proportional to the age of a woman and thus, it is advisable for oocyte cryopreservation to be performed at younger age. With the increasing awareness among the society of young women, oocyte cryopreservation is perceived to be mostly performed for women with the intention of social freezing. However, the data collected in our centre is contradicting with the perception.

**Aim:** To review the reasons of oocyte cryopreservation and subsequent effects towards clinical pregnancy rate (CPR) per return patient and per embryo transfer (ET) done.

**Methods:** A retrospective review of total 118 cases of oocyte cryopreservation in our centre from 2015-2020 were conducted. Two main reasons of oocyte cryopreservation were identified. The percentage of patients who returned and underwent ICSI after oocytes thawing were being recorded. CPR per patient and CPR per ET were being analyzed.

**Results:** Out of the 118 cases, 51 cases were social freezing while 67 cases were due to difficulties encountered during semen sample collection on the OPU day. From the social freezing group, two patients (3.9%) returned for ICSI procedure within 5 years, but no pregnancy was achieved. In contrast, for patients whereby the oocytes were frozen due to unavailable of sperm sample, 62.7% (n=67) of patients returned for oocytes to be warmed and utilized. The CPR per patient and CPR per ET is 21.3% (n=42) and 36.0% (n=25).

**Conclusions:** The CPR per patient and CPR per ET by warming oocytes for ICSI procedure is 21.3% and 36.0% respectively. As oocyte cryopreservation were mainly performed as emergency due to unplanned medical reason in our centre, and it shows slightly compromised and biased results compared to those treated using fresh oocytes. Whereas for social egg freezing, the actual treatment prospect will take a much longer time to establish.

### Conflict of Interest

May Chee Fam- N/A; Shi Yee Chen-N/A; Yin Thien Tan-N/A; Sze Tian Tee-N/A



## **SPERM DNA FRAGMENTATION FOR EVALUATION OF MALE INFERTILITY: A SINGLE CENTRE EXPERIENCE**

Deventhiran, Radha Krishnan<sup>1</sup>

<sup>1</sup>Dr. Geetha Haripiriya

### **ABSTRACT**

**Introduction:** Sperm DNA Fragmentation is a useful method for the suitable sperm for use of ART methods.

In this Cohort retrospective study, 104 male patients data was collected from Prashanth Fertility Research Centre in Chennai, India and their age ranged from 25 to 32 years. Among, 57 patient's data been appropriate for the study and 47 patients' data did not fulfill the criteria (Inclusion criteria). Samples are collected from the duration of Dec. 2017 to Feb. 2019. The Sperm DNA Fragmentation was done with the halo kit method. Also, FSH, LH, Testosterone values were taken along with other routine parameters. The data were presented in mean  $\pm$  SD and percentages.

**Materials and methods:** Assess the semen sample for its concentration, dilute the semen sample with culture medium to 5 to 10M/ml, and melt the agarose to the sperm chroma warmer that is maintained at 37°C and wait for 5 minutes, add 25 $\mu$ l of the semen sample to agarose and mix well, place the sperm cell suspension immediately onto the pretreated slides and place the cover slip avoid formation of bubbles, leave the slide at 4°C for 5 minutes. After 5 minutes, remove the cover glass into the slide carefully by sliding it. Incubate the slides horizontally in different solution with different time duration and drain it and stain it gently wash with distilled water and dry at room temperature.

**Results and Conclusions:** The Sperm DNA fragmentation was compared among the four groups. In the group, I (Excellent to good DNA Integrity) & group II (Good to fair DNA integrity), 100% & 90.9% pregnancy rate has been observed respectively. In group III (Fair to poor DNA integrity), 82.7 % has a successful pregnancy rate. There was no significant association between FSH, LH, Testosterone and sperm DNA fragmentation.

**Keywords:** Assisted Reproductive Technology (ART), seminal parameters, Sperm DNA fragmentation, Halo kit method.

### **Conflict of Interest**

Dr. Geetha Haripriya COI, Dr. Natarajan COI, Dr. Kumaresan Ramanathan COI

## **SPONTANEOUS HEMOPERTONEIUM IN PREGNANCY CONCEIVED BY ASSISTED REPRODUCTIVE TECHNOLOGY**

Ibonia, Kristine Andrea<sup>1</sup>; Cabling, Maria Lourdes<sup>1</sup>; Ibonia, Kristine Andrea<sup>1</sup>

<sup>1</sup>Brokenshire Hospital

### **ABSTRACT**

This is a case report of a 38 year old patient managed as a case of spontaneous hemoperitoneum in pregnancy (SHiP).

The couple had been desirous of pregnancy for 6 years, undergoing various fertility workup during the same period of time. The husband was diagnosed with azoospermia and underwent testicular biopsy which yielded a minute number of sperm cells. The patient then conceived after Intracytoplasmic Sperm Injection and in vitro fertilization, a dichorionic, diamniotic twin gestation.

At 33 weeks gestation, she was admitted due to sudden intense abdominal pain with profuse vaginal bleeding. Abruptio placenta was suspected and emergency cesarean section was done. Massive hemoperitoneum was noted. On inspection, actively bleeding, coalesced friable pinkish blebs with a pulsating bleeding vessel were seen at the left posteromidlateral corpus. Bilateral uterine artery ligation and repair of the bleeding site were done. Both babies were delivered at 35 weeks pediatric aging. Postoperative course was stable and both the mother and the babies were discharged.

SHiP is a relatively rare disorder associated with high morbidity and mortality. It is an unprovoked peritoneal bleeding reported to occur during the second half of pregnancy, in labor and early postpartum. Typical presenting symptoms are sudden intense abdominal pain and hypovolemic shock without revealed bleeding. The diagnosis of SHiP is rarely made before laparotomy and surgery is often due to a preoperative diagnosis of abruptio placenta, as what has occurred in this case. Etiology of SHiP remains unknown. It may be multifactorial, but has been reported in women with endometriosis and decidualosis including those of patients with diffuse decidualization linked to controlled ovarian hyperstimulation and embryo transfer.

This case presents a life-threatening event for both the mother and her babies which needed immediate diagnosis and surgical intervention. Further studies are needed to understand SHiP to improve perinatal outcome.

### **Conflict of Interest**

Kristine Andrea Ibonia N/A; Maria Lourdes Cabling N/A

## STUDYING THE EPIGENETIC SIGNALLING USING EPIGENETIC MODIFIER AND EFFECT ON DEVELOPMENTAL COMPETENCE OF IN-VITRO CLONED MAMMALIAN EMBRYOS

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<sup>1</sup>National Dairy Research Institute

### ABSTRACT

**Introduction:** There is accumulating evidence that histone modifications signals genes to switch ON and OFF through promoter DNA methylation. Epigenetic regulation involves post translational histone modifications and DNA methylation. Histone modifications play key role during epigenetic reprogramming in mammalian embryo development guiding zygote to achieve pluripotent state and differentiation of cell lineages later. Aberrant epigenetic reprogramming leads to low developmental competence of cloned goat (*Capra hircus*) embryos *in-vitro*. Improper erasure of H3K9me2 epigenetic mark halts the cloned embryo growth. H3K9me2 hypermethylation associates with DNA hypermethylation in promoter regions of pluripotency genes i.e. POU5f1, SOX2 and NANOG which leads to inactive transcription of these genes resulting in low embryo division potential. In this study, an epigenetic modifier i.e. BIX01294, a quinazolinamine derivative is used to study epigenetic signalling known to decrease H3K9me2/3 levels in somatic cell reprogramming and increase the developmental competence of cloned mammalian embryos.

**Material and methods:** Hand-made cloning, Goat fetal fibroblast cell culture, qPCR, Immunocytochemistry, Global DNA methylation ELISA

**Results:** Significant increase was observed in BIX01294 treated cloned embryos ( $16.53\% \pm 0.2$ ) compared to control group ( $6.53\% \pm 0.2$ ) at zygotic genome activation and morula stage i.e. BIX treated ( $9.61\% \pm 0.33$ ) compared to the control group ( $3.15\% \pm 0.27$ ). Immunostaining fluorescent intensity analysis of H3K9me2 mark shows significant reduction in BIX treated goat fetal fibroblasts cells ( $6.72 \pm 0.49$ ) compared to control group ( $21.64 \pm 1.35$ ). Significant reduction in global DNA methylation observed in BIX treated fetal fibroblasts cells to untreated cells i.e. 0.49% vs. 0.91% respectively in global DNA methylation ELISA analysis in observing regulation of DNA methylation by histone methylation. Relative mRNA abundance of pluripotency genes i.e. Pou5f1 and Sox2 was significantly higher as compared to untreated and IVF embryos with  $p < 0.05$ .

**Conclusions:** H3K9me2 regulates pluripotency genes transcription through DNA methylation and decrease in H3K9me2 levels results in increase in developmental competence of cloned goat embryos.

### Conflict of Interest

Amit Kumar N/A, Abhishek Thakur N/A, Shavi Verma N/A, Sikander Saini N/A, Satish Kumar N/A, Dhruva Malakar N/A

# SUGGESTED CO-OPTIMIZATION OF ULTRALOW OXYGEN AND FREQUENT MEDIA CHANGE TO ENABLE ESET: LESSONS FROM EM-BRYONIC STEM CELLS WHERE FREQUENT MEDIUM CHANGING DECREASES G1 CELL-CYCLE DELAYS AS CELLS ENTER WARBURG ANABOLISM

Rappolee, Daniel<sup>1</sup>; Hsieh, Tzu-Bou<sup>1</sup>; Sethuram, Ramya<sup>1</sup>; Abdulhasan, Mohammed<sup>1</sup>; Ruden, Ximena<sup>1</sup>; Ruden, Douglas<sup>1</sup>; Awonuga, Awoniyi<sup>1</sup>; Takayama, Shuichi<sup>2</sup>; You, Yuan<sup>1</sup>; Alvero, Ayesha<sup>1</sup>; Puscheck, Elizabeth<sup>1</sup>

<sup>1</sup>Wayne State University Medical School, <sup>2</sup>Georgia Tech University

## ABSTRACT

**Introduction:** Human preimplantation culture for single blastocyst transfer is suboptimal: IVF blastocyst formation rate is ~50% with even lower implantation and birth rates. Culture optimization should improve blastocyst and pregnancy rates. Media is changed infrequently, and labs improving blastocyst rates with ultralow (2%) O<sub>2</sub> don't change media. Here we model development of embryonic stem cells (ESC) from blastocysts to show that lactate-producing Warburg metabolism increases with time, and media change is important to prevent G1 cell cycle delays.

**Methods:** Fluorescence ubiquitinated Cell-cycle indicator (FUCCI) mouse ESC were cultured 72hr in a live imager where fluorescent green ESC are in S-G2-M-phase, and non-Green are in G1 phase of cell cycle. ESC cultured at 20% O<sub>2</sub> were recorded for confluence and number of green cells every 2hr where media was changed with two frequencies: every 24hr or 12hr. After 72hr culture, ESC cultured with 24hr frequency were assayed by RNAseq using Illumina NovaSeq6000 for bulk transcriptome or single cells (sc)RNAs after 10X Genomics cell sorting. In RNAseq studies media components were changed to emulate normal stemness and development.

**Results:** FUCCI ESC confluence/growth is a sigmoidal, exponential curve with 24hr media changes but surprisingly green FUCCI ESC decrease and then increase 5-7fold before and after media change: suggesting G1 delay. This amplitude of G1 delay decreases significantly with 12hr medium change although growth rate didn't increase, perhaps due to confounding variables. The transcriptome of 24hr group showed ESC moving from Naïve to Formative pluripotency during culture (E3.5/4.5 ICM to E5.5 progression), and the expression of 18 Warburg genes increase 2fold during this development.

**Discussion:** Transcriptomic studies confirm reported low mitochondrial charge and high lactate starting at blastocyst and increasing after. Previous reports showed optimization of trophoblast stem cells to near-in vivo growth at 2% O<sub>2</sub>, but morbidity instead without 12hr feeding-frequency. Together, data suggest blastocyst-derived cell culture will optimize at 2% with necessary frequent media change.

## Conflict of Interest

Tzu-Bou Hsieh N/A, Ramya Sethuram N/A, Mohammed Abdulhasan N/A, Ximena Ruden N/A, Douglas M. Ruden N/A, Awoniyi O Awonuga N/A, Shuichi Takayama N/A, Yuan You N/A, Ayesha Alvero N/A, Elizabeth E. Puscheck N/A, and Daniel A. Rappolee N/A

## SUMO2, A SMALL UBIQUITIN-LIKE MODIFIER, IS ESSENTIAL FOR DEVELOPMENT OF MURINE PREIMPLANTATION EMBRYOS

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<sup>1</sup>National Key R&D Program of China, <sup>2</sup>Natural Science Foundation of Qinghai Province, China, <sup>3</sup>Natural Science Foundation of Shaanxi Province, China, <sup>4</sup>College of Veterinary Medicine, Northwest A&F University, <sup>5</sup>Academy of Animal Science and Veterinary Medicine, Qinghai University

### ABSTRACT

**Introduction:** Small ubiquitin-like modifier 2 (SUMO2) is a small protein that modulates the stability and activity of other proteins. Although a variety of activities have been attributed to SUMO2, its function in preimplantation embryos is still obscure. Here we demonstrated that knockdown of SUMO2 will cause obstacles to blastocyst development and abnormal lineage differentiation.

**Material and methods:** siRNA microinjection, immunofluorescence (IF) staining and quantitative real-time PCR (qRT-PCR)

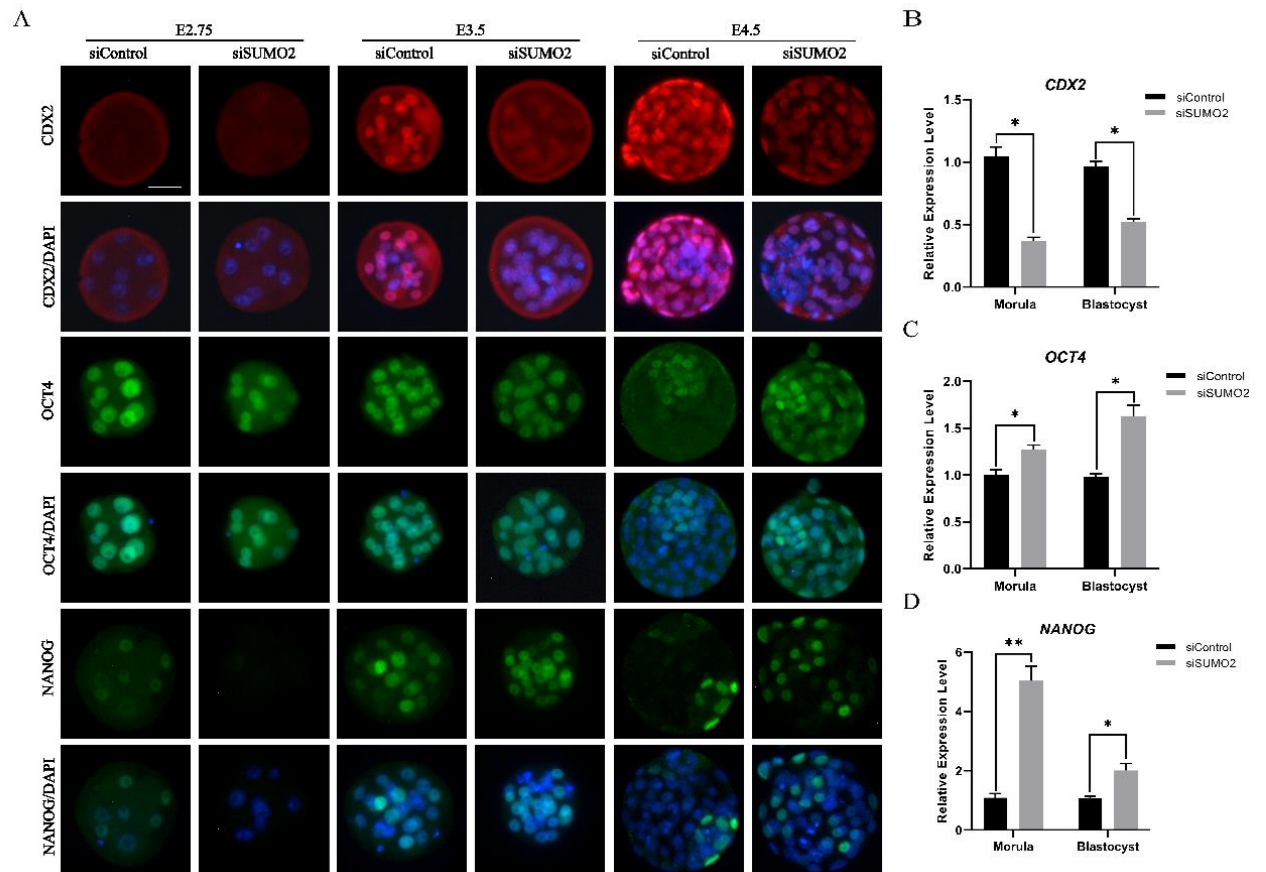
**Results:** We first explored the expression of SUMO2 protein in early embryos, and showed that compared with the 2-cell stage, the expression was increased at first, peaked at the 8-cell stage, and then dramatically decreased. To study the function of SUMO2, we used siRNA microinjection to knock down SUMO2. The silencing of SUMO2 significantly reduced the rate of in vitro blastocyst development from 75.56% to 40.60%. Notably, knockdown of SUMO2 (KD) altered the expression of CDX2, OCT4, and NANOG. The number of cells expressing CDX2 decreased, while OCT4 and NANOG were ectopically expressed in siSUMO2 embryos.

*(image)*

(A) Representative images of CDX2, OCT4 and NANOG immunofluorescence staining after injection with siControl and siSUMO2 at E2.75, E3.5, and E4.5. Scale bar = 30  $\mu$ m. (B-D) Relative mRNA expression of CDX2 at the morula stage and in blastocysts in the experimental group compared to controls.

**Conclusions:** In summary, the present study demonstrates that silencing SUMO2 caused a barrier to lineage differentiation. SUMO2 plays a significant role in preimplantation development of mouse embryos probably through regulating pluripotency genes.

### Abstract image



### Conflict of Interest

Ying Yang, Yingbing Zhang, Peipei Qiao, Bin Yang, Huiqun Jia, Yong Zhang, Jun Zhang, and Jianmin Su\*

## THE 1ST POLAR BODY TRANSFER: THE EFFECTIVENESS OF TECHNIQUE IN DOUBLING OOCYTES

Gontar, Julia<sup>1</sup>; Buderatska, Nataliia<sup>1</sup>; Lavrynenko, Sergiy<sup>1</sup>; Parnitska, Olga<sup>1</sup>; Kazachkova, Nadiya<sup>1</sup>; Ilyin, Igor<sup>1</sup>

<sup>1</sup>Medical Center IGR

### ABSTRACT

**Introduction:** The transfer of the patient's 1st polar body(PB) to donor oocyte cytoplasm has proven to be an effective method to help patients with low ovarian reserve and poor response. This technique allows getting high quality blastocysts(HQB) from modified oocytes in addition to embryos derived from maternal gametes. In confirmation of this, three healthy babies have been born to this day thanks to this technique.

**Materials and Methods:** The study was performed in the Medical Center IGR from 2017 to 2021 and involved 361 oocytes (group A) obtained from 68 patients (mean age  $38.4 \pm 6.4$  years) and 334 oocytes (group B) from 47 donors (mean age  $27.0 \pm 3.6$ ). The procedure was made using Nikon TiEclipse(Japan) inverted microscope, Saturn3 laser console(UK). Preimplantation genetic testing for aneuploidy (PGT-A) was performed using trophoctoderm(TE) biopsy that were diagnosed by NGS (Ion S5,USA). Statistical analysis was carried out using Shapiro-Wilk test for normality and Chi-square test.

**Results:** The total number of oocytes in this study comprised 695 cells. The mean numbers of original oocytes per patient were  $4.2 \pm 2.7$  and modified oocytes were  $3.9 \pm 2.2$ . Thereby, aggregated amount of oocytes suitable for the fertilization has grown up to  $8.2 \pm 4.7$  per patient. In the group A there were 93 HQB (25.8%) that formed from 361 original patients' oocytes and 70 HQB (20.9%) developed from 334 modified oocytes (group B). The statistically significant difference(SSD) was not found between the groups ( $p > 0.05$ ). 67 blastocysts from group A and 48 blastocysts from group B were biopsied for PGT-A. The number of euploid embryos was 19 (28.4%) and 15 (31.3%) in the group A and group B, respectively, without SSD.

**Conclusions:** Research shows that use of the 1<sup>st</sup> PB transfer technique increases the yield of high qualitative blastocysts at least by 14,5%. This technique is useful to increase the number of female germ cells and avoid the full oocytes donation.

### Conflict of Interest

Julia Gontar N/A, Nataliia Buderatska N/A, Sergiy Lavrynenko N/A, Olga ParnitskaN/A, Nadyia Kazachkova N/A, Igor Ilyin N/A

## THE EFFECT OF INTRAUTERINE ADMINISTRATION OF HUMAN CHORIONIC GONADOTROPIN (HCG) ON INTRA UTERINE INSEMINATION (IUI) OUTCOME: A RANDOMIZED PROSPECTIVE STUDY

Fakih, Chadi<sup>1</sup>; Mourad, Youmna<sup>1</sup>; Raad, George<sup>1</sup>; Zahwe, Ranine<sup>1</sup>; Mourad, Jana<sup>2</sup>; Lakkis, Lara<sup>2</sup>; Chamas, Tatiana<sup>2</sup>; Fakih, Ibrahim<sup>1</sup>; Fakih, Ghada<sup>1</sup>; Samad, Sarab<sup>3</sup>; Saad, Jacqueline<sup>3</sup>; Fakih, Fadi<sup>1</sup>  
<sup>1</sup>Al Hadi Laboratory and Medical Center, <sup>2</sup>Lebanese University, <sup>3</sup>Balamand University

### ABSTRACT

**Background:** Embryo successful implantation depends on a complex embryo-maternal crosstalk. Aside from euploidy, the receptivity of the endometrium is most crucial for successful implantation. Human chorionic gonadotropin is a major player in implantation, being involved in decidualization, trophoblastic invasion, proliferation of uterine natural killer cells, endometrial angiogenesis, maintenance of progesterone secretion and a shift in endometrial gene expression. The addition of hCG to semen preparation media before IUI has not yet been studied.

**Methods:** The study was a triple-blind randomized clinical trial (RCT) performed from the first of March 2018 to the end of February 2019 at AL HADI MEDICAL CENTER. A total of 210 women were randomized into three groups, A, B and C, using computer generated random numbers. Group A patients received intrauterine hCG dose of 500 IU (Choriomon from IBSA) at the time of insemination while group B patients received a dose of 1000 IU at the time of insemination. No hCG were added to group C.

**Results:** Patients' demographic and baseline characteristics were comparable. We had statistical difference in clinical pregnancy (p value 0.0471, 15.94% in group A, 15.15% group B, 9.23% group C), the number of implanted sac (p value 0.0283, 24.68% in group A, 15.28% in group B, 10.45% in group C), and live birth rate (p value 0.083, 14.29% in group A, 13.89% in group B, 5.97% in group C). But no statistically significant differences between the 3 groups regarding the miscarriage rate (p value 0.297, 6.49% in group A, 1.39% in group B, 4.48% in group C).

**Conclusion:** Adding HCG to the semen preparation media before IUI increases its effectiveness on the pregnancy rate, live birth rate and number of implanted sacs without increasing miscarriage rate, the result that will form a landmark in IUI practice.

### Conflict of Interest

Chadi Fakih (N/A), Youmna Mourad (N/A), Georges Raad (N/A), Ranine Zahwe (N/A), Jana Mourad (N/A), Lara Lakkis (N/A), Tatiana Chamas (N/A), Ibrahim Fakih (N/A), Ghada Fakih (N/A), Sarab Samad (N/A), Jacqueline Saad (N/A), Fadi Fakih (N/A)



# THE EFFECT OF TIMING OF OOCYTE DENUDATION FROM OOCYTE RETRIEVAL IN THE TOTAL FERTILIZATION FAILURE (TFF) AMONG IN VITRO FERTILIZATION-INTRACYTOPLASMIC SPERM INJECTION (IVF-ICSI) CYCLES

Bermio, Gladys Anne<sup>1</sup>

<sup>1</sup>ACE Medical Center Quezon City

## ABSTRACT

**Background and aim:** With the advancement in ART, fertilization and pregnancy rates in IVF continue to improve. Microinjection of viable sperm theoretically alleviates male contribution to infertility. However, even with ICSI, total fertilization failure (TFF) continues to persist. This study aims to evaluate the effect of oocyte incubation after retrieval in TFF among IVF-ICSI cycles and to identify factors affecting TFF.

**Methods:** This is a retrospective cohort study involving 995 stimulated cycles using the antagonist protocol, excluding ICSI from surgically retrieved sperm. Samples were clustered into three timings of oocyte denudation from retrieval: Group 1: <1hour ( $n=393$ ), Group 2:  $\geq 1$ hour to <2hours ( $n=260$ ) and Group 3:  $\geq 2$ hours ( $n=342$ ). Other variables considered were etiology of infertility, female age, days of stimulation and total number of oocytes retrieved. TFF across groups were determined and analyzed.

**Results:** Overall TFF was 4.5%. TFF among groups were 4.8%, 5.8% and 3.2%, respectively. Female age and days of stimulation were similar among groups (36.3, 36.8, 36.4 years and 10.9, 11, 11 days), while the total number of oocytes retrieved were significantly highest in Group 3 (11.3 vs 9.5 and 8.7 in Groups 2 and 3,  $p<0.001$ ) and the distribution of etiology of infertility was significantly varied ( $p<0.002$ ). Multiple logistic regression analysis showed that incubation of oocyte prior to denudation for at least 2 hours tend to decrease TFF rate incidence, however, was not statistically significant. Among the etiologies of infertility, male factor seemed to increase the incidence of TFF. Total number of oocytes significantly affect the occurrence of TFF, decreasing its incidence by 35.7% for every single increase in oocyte number ( $p<0.001$ ).

**Conclusion:** Timing of incubation of oocyte did not significantly affect the occurrence of TFF. Among factors studied, male factor infertility and a low number of oocytes adversely affect TFF.

## Conflict of Interest

N/A

## THE IDENTIFICATION OF GENETIC MARKERS COMPLEX AND CEATION OF DESIGN OF UNEXPLAINED INFERTILITY BY USING NEXT – GENERATION SEQUENCING (NGS) SYSTEM

Jasinskiene, Egle<sup>1</sup>

<sup>1</sup>Marija Caplinskiene, Prof., Vilnius, Lithuania, m.caplinskiene@gmail.com

### ABSTRACT

**Introduction:** Infertility is one of the most important reproductive health problems in Lithuania. Unexplained infertility is one of the main causes of infertility. It is estimated to account for 25%. Analysis of genetic markers using the latest diagnostic systems as a next-generation sequencing (NGS) system, identification of these markers is very important in the assessment of risk factors and causal etiopathogenesis mechanisms in the solution of infertility problems.

**Objective:** To determine genetic markers those are related or may be associated with unexplained infertility. To create diagnostic panel “Unexplained Infertility” that could be applied in daily clinical practice.

**Methods:** A systematic review of the literature is performed to analyze genes that are related or may be associated with unexplained infertility. The review was conducted in the electronic scientific databases PUBMED, Medline, and Cochrane, including articles published since 2010 until 2020. The following keywords were used for the search: "genes", "unexplained infertility", "diagnostic panel", "and next- generation sequencing (NGS)".

**Results:** According to the literature and research, these diagnostic panels have been developed:

Gene sets – female unexplained infertility: MTHFR, FOLR3, TCN2, CTH, SLC19A1, SYCP3, ENOS, CYP19A1, ESR1, LIF, HABP2, MLH3, TUBB8, ZP1, PAD16, TLE6, F2, CFTR, CAPN10, AR, FSH, LHCGR, ACE, PAI-1-675, MnSODA16V, RNLS, VDR, PALT2.

Gene sets – male unexplained infertility: CATSPER1, POLG, RPL23A, RPS27A, RPS3, RPS8, TOMM7, MTHFR C677T, APLF, CYB5R4, ERCC4, TNFRSF21, MORC1, PIWIL1, ZFAND6, RBMY1F, DPY19L2, ADAM3A, NXF2, SIRPB1, FSHR, LHCGR, AR.

**Conclusions:** The correctly established infertility diagnosis helps to manage individual treatment. The diagnostic panel “Unexplained Infertility” should be included in the routine testing, determining the cause of unexplained infertility, applying new diagnostic tests and will have practical application in solving of unknown etiology of infertility determination and indicating the correct future medicine interventions to solve the growing problem.

**Key words:** Unexplained infertility; Genetic markers; Next-generation sequencing (NGS); Lithuania.

### Conflict of Interest

Egle Jasinskiene, Vytautas Magnus University, Vileikos 8-306, Kaunas, Lithuania. Marija Caplinskiene, Vytautas Magnus University, Vileikos 8-306, Kaunas, Lithuania

## THE IMPACT OF BODY MASS INDEX ON OVARIAN STIMULATION FOR ART.

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### ABSTRACT

**Introduction:** Overweight and obesity are well-known risk factors for female fertility, but their impacts on Assisted Reproduction Techniques (ART) are still controversial. This study aimed to evaluate the impact of Body Mass Index (BMI) on ovarian stimulation for ART.

**Material and Methods:** A total of 1835 In vitro fertilization (IVF) cycles conducted between 2013 and 2020 were included for ovarian response analysis. Patients were divided into three groups according to BMI (kg/m<sup>2</sup>): Group 1 <25 (n=1332); Group 2 25-29.9 (n=373) and Group 3 ≥30 (n=130). The cancelled IVF cycles rate, the dose of gonadotropins used, number of retrieved oocytes, and oocyte maturity rate were analysed. The statistical analyses were made using ANOVA, Chi-square test, multiple logistic regression, and post-hoc curve, and generalized estimating equations were performed to consider patients and cycles, considering p<0.05 statistically significant.

**Results:** A significant linear tendency to a higher proportion of cancelled IVF cycles as higher as was the BMI was observed (6.9% vs. 7.8% vs. 10.4%, p=0.002). We also found a higher gonadotropin's total dose (UI) in groups 2 and 3 (1685±595 vs. 1779±610 vs. 1805±563, p=0.001). A greater number of mature oocytes was observed in Group 1 and 2 (6 [6.4-7] vs 6 [5.6-6.6] vs 4 [4.6-6.7], p=0.0111), but no difference between oocyte mature rate (%) was found (80 [76-78.6] vs 80 [74.3-79.7] vs 77.7 [71.4-81], p=0.877).

**Conclusions:** Overweight and obesity have a negative impact on the ovarian response to stimulation. The higher proportion of cancelled IVF cycles, found higher in overweight and obesity groups, may have been related to the same poor ovarian response that required higher gonadotropin doses. This study shows that even the best overweight and obese ovarian responders had a worse response when compared to eutrophic patients.

### Conflict of Interest

Victoria Dornelles N/A, Marta Ribeiro Hentschke N/A, Isadora Badalotti Telöken N/A, Natália Fontoura de Vasconcelos N/A, Bibiana Cunegatto N/A, Vanessa Devens Trindade N/A, Álvaro Petracco N/A, Alexandre Padoin N/A, Bartira E. Pinheiro da Costa N/A, Mariangela Badalotti N/A

## THE RELATIONSHIP BETWEEN SEMEN PARAMETERS, PATERNAL AGE AND DNA FRAGMENTATION LEVEL

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### ABSTRACT

**Introduction:** To explore the relationship between paternal age, semen parameters and DNA fragmentation; and to evaluate is there any impact of density gradient centrifugation (DGC) on sperm DNA integrity.

**Material and methods:** A retrospective review of 90 men who had sperm DNA fragmentation (SDF) test from Sep 2020 to Feb 2021 at fertility unit of Thomson hospital. Cases with low sperm count (<5 million/mL), frozen-thawed and surgically retrieved sperm were excluded from study. Semen sample was analyzed according to WHO 5<sup>th</sup> edition. Both pre-wash and post-wash semen samples (density gradient centrifugation) were analyzed for SDF by sperm chromatin dispersion test (Halosperm® G2, Halotech). SDF level was calculated based on 300 sperms and expressed as DNA fragmentation index (DFI).

$$\text{DFI (\%)} = \frac{\text{No. of spermatozoa with fragmented DNA}}{\text{No. of spermatozoa counted}} \times 100$$

The relationship between semen parameters, paternal age and DFI was assessed using Pearson's correlation coefficient (r). Wilcoxon signed rank test was used to compare the difference between pre-wash and post-wash sample. P<0.05 was considered statistically significant.

**Results:** A significant moderate inverse relationship was observed between sperm motility and DFI (r= -0.323, p=0.002). Sperm concentration was weakly correlated with DFI (r= -0.2272, p=0.031). There was no correlation between DFI and paternal age (r= 0.0466, p=0.671); DFI and morphology (r= -0.0471, p=0.066). DFI of post-wash sample was significantly lower compared with pre-wash sample (2.9±2.6 vs 11.35±8.2, p=0.0008).

**Conclusions:** There is moderate correlation between SDF and sperm motility. DNA fragmentation may serve as adjunct test to semen analysis. However, randomised controlled trial is required to confirm the clinical value of DNA fragmentation test, as well as which group of patients should be offered and benefit most from this test. Our study also showed that sperm processing by DGC do not lead to higher DNA fragmentation. Instead, SDF is significantly improved after processing.

### Conflict of Interest

ShiYee Chen (N/A), JiGin Ku (N/A), KaiBoon Yong (N/A), CheeSan Heong (N/A)

# THE UPTAKE AND PERFORMANCE OF WHO 5TH EDITION MANUAL CLASSIFICATION SYSTEM TO ASSESS SPERM MORPHOLOGY SINCE 2010: EXPERIENCE FROM AN AUSTRALIAN EXTERNAL QUALITY ASSURANCE (EQA) PROGRAMME.

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<sup>1</sup>Edith Cowan University, <sup>2</sup>EQASRM

## ABSTRACT

A shift in sperm morphology assessment over the years, from early liberal criteria to contemporary strict criteria, culminated in the latest WHO 5<sup>th</sup> Edition (WHO5) in 2010. EQA programmes provide opportunity to assess the uptake and performance of laboratories using WHO5.

Samples were posted to enrolled laboratories each quarter (EQASRM; Northlands, Western Australia, Australia). Each quarterly distribution included 3 “wet” semen samples and a pre-stained slide. The proportion of sperm with normal morphology for each sample was submitted online, and statistical summaries (group mean and standard deviation) per classification method made available. Results for the semen samples only were reviewed from sample M209 (2010-2011) through to sample M375 (2020-2021), representing 42 distributions.

In 2009-2010, poor standardization was revealed by a range of morphology classification systems used by the 84 enrolled laboratories (WHO4, 60.7% laboratories; WHO3, 18.0%; WHO2, 5.9%; strict, 13.1%; others, 2.4%). The released WHO5 morphology classification system was rapidly taken up; 55/76 (72.4%) laboratories in 2012-2013 and 51/55 (94.4%) laboratories in 2020-2021. There was a steady improvement in between-laboratory variation, as shown by reduced CVs over time, with a negative correlation ( $r = -0.7279$ ,  $p < 0.00001$ ) when the CVs were plotted against the distribution number. Despite this improved precision, laboratories became stricter over time with the reported normal forms going down as the distribution number went up ( $r = -0.7765$ ,  $p < 0.00001$ ).

In conclusion, (i) the introduction of WHO5 resulted in the effective adoption of its morphology classification system, (ii) laboratories showed improved between-laboratory variation over time, (iii) the identification of normal forms over time was inconsistent as laboratories became more strict, and (iv) given the reduction in reported normal forms over time when working from the same WHO5 manual, it appears that either increased training of laboratory personnel or the consideration of validated objective automated analysers would seem warranted.

## Conflict of Interest

Michelle Kitson N/A, Dr. Phillip Matson N/A, Emily Zuvela N/A

## **USE OF HIGHLY PURIFIED HUMAN MENOPAUSAL GONADOTROPIN (HP-hMG) FOR CONTROLLED OVARIAN STIMULATION (COS) IN REAL-LIFE: INTEREST OF THE AMH (THE AME STUDY).**

Bernot, Mathilde<sup>1</sup>

<sup>1</sup>NA

### **ABSTRACT**

The individual response to controlled ovarian stimulation (COS) depends on several factors. The relationship between AMH level and the number of recruitable follicles has been established. However, in practice the dose of gonadotropins is not always adjusted linearly to the level of AMH.

French non-interventional, longitudinal, prospective, multicenter, cohort study conducted on women initiating HP-hMG 600 IU/mL for COS within their first IVF/ICSI cycle. Data were collected from COS initiation up to 10-11 weeks after embryo transfer. The primary objective was to evaluate the relationship between AMH level and the initial dose of HP-hMG. The study involved 25 French ART public and private centers. Patient recruited were infertile women aged between 18 and 42 years old undergoing their first IVF/ICSI cycle for whom a treatment with HP-hMG 600 IU/mL was initiated.

297 patients were enrolled in the study, 235 of whom compose the per protocol population. The median age was 33 years and mean BMI was  $24.3 \pm 4.8$  kg/m<sup>2</sup>. The mean AMH level was  $2.3 \pm 1.7$  ng/mL and the mean AFC was  $16.0 \pm 8.4$ . Antagonist protocol was used for down regulation in 84.3% of the cycles. The mean initial and total doses of HP-hMG were  $234 \pm 73$  IU/mL and  $2473 \pm 971$  IU/mL respectively. Statistically significant negative correlations between the serum AMH level and the initial/total doses of HP-hMG ( $p < 0.001$ ) were shown. 94.0% of the women had obtained embryo(s) and fresh embryo transfer was performed in 72.8%. The clinical pregnancy rate was 28.5% per cycle and no statistically significant difference was observed according to serum AMH level. Among the safety population (N=258), three patients (1.2%) experienced ovarian hyperstimulation that led to hospitalization.

AMH level impacts the initial/total dose of HP-hMG administrated: the lower the AMH, the higher the initial/total dose of HP-hMG ( $p < 0.001$ ). The weight did not influence the initial dose, but clearly impacts the total dose thus suggesting a dose adaptation.

### **Conflict of Interest**

Porcu-Buisson, G. (personal fees from Ferring SAS, during the conduct of the study); Hamamah, S. (N/A); Avril, C. (personal fees from Ferring SAS, during the conduct of the study); Barrière, P. (personal fees from Ferring SAS, during the conduct of the study)

## USING INSULIN-LIKE GROWTH FACTOR - 1 FOR IN VITRO MATURATION OF HUMAN GV OOCYTES IN THE PRESENCE OF CUMULUS CELLS

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### ABSTRACT

**Introduction:** The study of regulation mechanisms of oocyte maturation is necessary to improve infertility treatment efficiency by assisted reproductive technologies. The aim of this work was to assess the maturation and fertilization rates of oocytes at the *germinal vesicle* (GV) stage in the presence of cumulus cells and insulin-like growth factor-1 (IGF-1).

**Material and methods:** GV oocytes (n = 113) were obtained from patients (31 ± 4.6 years old) after controlled ovarian stimulation performed with recombinant follicle stimulating hormone with antagonists gonadotropin-releasing-hormone (GnRH). Agonist GnRH was used as a trigger for the final follicle maturation. Oocyte maturity was assessed after removal of cumulus cells after preliminary exposure to hyaluronidase. For maturation, GV oocytes were placed in Global for fertilization medium (group 1), with supplementation of autologous cumulus cells (group 2) and with supplementation of autologous cumulus cells and IGF-1 at concentration of 50 ng/ml (group 3), 100 ng/ml (group 4), 200 ng/ml (group 5). The gamete maturation was assessed by the presence of the first polar body after 24-48 h. Oocytes at the metaphase II stage were fertilized by ICSI and fertilization rate was assessed in 18 hours.

**Results:** It was showed that the highest maturation and fertilization rates was in group 5 and significantly differed from groups 1 (p<0.01) and 2-4 (p<0.05). It should be noted that in groups 4 and 5, cumulus cells formed sphere-like structures with close contacts between themselves and oocytes in contrast to groups 2 and 3.

**Conclusions:** The contact loss between cumulus cells and the oocyte as a result of denudation negatively affects further maturation and fertilization. The addition of IGF-1 (200 ng / ml) to the culture medium allows partially to restore the lost contacts which probably led to an increase in the maturation rate 84% and fertilization rate of 86.7% of GV oocytes.

### Conflict of Interest

Yurchuk T - N/A, Piniayev V- N/A, Petrushko M - N/A.

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