

## P-01. The use of hysteroscopy in patients with fertility disorders

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**Objective:** To characterize patients with fertility disorders, to which a hysteroscopy was performed in order to assess their endometrial cavity. Hysteroscopy is a clinical procedure allowing a gynecologist to observe the inner part of the uterus by means of an endoscopy. This procedure can be made with diagnostic purpose or as a surgical intervention method.

**Methods:** Design: A retrospective descriptive study. Context: Assisted Reproduction Center for patients with infertility disorders. 92 women in a fertility study made the sample, from February 16<sup>th</sup> to May 18<sup>th</sup> 2018. A hysteroscopy procedure was carried out in 92 patients. For their analysis, the "SPSS 15.Mediciones" program was used. The procedure defined elements like age, personal pathological history, type of infertility, nutritional state, infertility cause, ultrasonographic finding, diagnosis prior to hysteroscopy, hysteroscopic finding, endometrial tissue histological results.

**Results:** The higher proportion among patients suffering from infertility was from 26 to 35 years (63%). The higher percent of studied patients was supposedly healthy. There was evidence that 59.8% had a normal weight, and 76.1% had a secondary infertility disorder. The most common cause of infertility was the tubal factor (52.1%). Abnormal finding of the endometrial cavity was observed in 54.4% of the cases. Regarding the results of the endometrium histological studies, it was diagnosed that in 42 out of 31 (70.4%) cases there were endometrial pathologies.

**Conclusions:** Hysteroscopy is a procedure to be carried out to all patients suffering from infertility disorders because it improves the evaluation of the endo-uterine cavity, and prevents implantation failures by endometrial pathology.

## P-02. Serum metabolomic profile as a non-invasive tool for the diagnosis of endometriosis-related infertility

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**Objective:** Despite the fact that endometriosis significantly reduces quality of life, endometriosis-induced pain can often be confused with menstrual cramps. Because a complete diagnosis requires laparoscopic, instead of establishing the diagnosis of endometriosis by invasive approaches, empirical treatments for pain may be prescribed, and the diagnosis is usually postponed. Nonsurgical methods for the diagnosis of endometriosis could be used as an adjunct tool for the selection of patients who must undergo laparoscopy to obtain a definitive diagnosis. In the post-genomic era, metabolomics has emerged as a powerful tool to identify biomarkers for given conditions in different types of biological samples, therefore the goal for the present study was to identify serum biomarker of endometriosis grades III and IV by mass spectrometry-based metabolomics.

**Methods:** This case-control study included serum samples from 100 patients <38 years undergoing intracytoplasmic sperm injection (ICSI), in a private university-affiliated IVF center. Samples collected from January/2017 to December/2017 were split into two groups according to the cause of infertility: the Endometriosis-Group (samples derived from patients with grade III and IV endometriosis, n=50) and the Control-Group (samples derived from patients with isolated male factor infertility, n=50). To validate the model, 30 other samples from women without any evidence of endometriosis (the disease-free group) were tested. The metabolomic profile of each sample was obtained by mass spectrometry. Partial least square discriminant analysis (PLS-DA) was applied to the dataset in order to determine the discriminatory components based upon the combination of variable influence on projection (VIP) values. These values were used to build a single receiver operating characteristic (ROC) curve.

**Results:** Except for the pregnancy rate, which was decreased in the Endometriosis-Group (32.0% vs. 72.0%, for Endometriosis and Control groups respectively,  $P=0.007$ ), the patient and cycle characteristics did not differ between groups. A total of 429 and 484 ions for the positive and negative ionization modes were analysed, respectively. Considering components one, two and three, the PLS-DA was able to clearly distinguish the Endometriosis-Group from the Control-Group for both positive and negative ionization modes. Ten potential biomarkers were selected based on their importance for model prediction, five in the positive and five in the negative ionization modes. These ions were used to build the ROC curve, which presented an area under the curve (AUC) of 0.904 (CI 95%: 0.796-0.985), indicating the accuracy of the biomarkers for sample classification in the Control or Endometriosis groups. Considering these ions as possible biomarkers, the model was able to correctly classify 84% of the patients and when the validation set was tested, the model was able to correctly classify 86.6% of the samples. Two metabolites were identified by the database. Triacylglycerols and alpha-amino acids were more abundant in serum of positive endometriosis patients, while the other ions were not identified by the currently available database.

**Conclusions:** Serum-metabolomics may be a valuable approach to the diagnosis of endometriosis. The value of the present work was proven by its high predictive power and accuracy. This was confirmed when 86.6% of the samples from the validation set was correctly classified. This technology may be used as an adjunct tool for the selection of patients who must undergo laparoscopy to obtain a definitive diagnosis.

### P-03. Semen quality difference between samples collected on the day of intra uterine insemination versus at the initial evaluation of male fertility

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**Objective:** To evaluate difference in semen parameters in samples collected for initial assessment of male fertility compared to samples collected on the day of intra uterine insemination (IUI).

**Methods:** We analyzed data of homologous IUI cycles carried out between January 2014 and December 2017. Cases with initial semen evaluation performed within 12 months prior to insemination treatment and the second evaluation made on the day of the insemination were included. Semen profiles were evaluated according WHO criteria, fifth edition. Student's T Test and Mann-Whitney U test were employed for statistical analysis, where parameters of semen volume (ml), sperm concentration ( $10^6$  per ml), total motility (progressive motility + non-progressive motility, percentage) and progressive motility (percentage) were compared between both samples. Data was also segmented by age range (<31, 31-34, 35-40, 41-55) for analysis. Variables were expressed as mean  $\pm$  standard error of the mean and a *p* value < 0.05 denotes significant differences.

**Results:** One hundred and fourteen cases were included with a male age range between 24-55 years ( $37.09 \pm 0.56$ ). Difference was found in the semen volume parameter between initial evaluation samples and samples collected the day of insemination, with a decrease in the latter ( $3.06 \pm 0.12$  vs.  $2.47 \pm 0.10$ ). Regarding sperm concentration, there was a difference between both samples in patients with an age range of 41-55 years, with a decrease of this parameter in the samples evaluated on the day of insemination ( $46.06 \pm 5.16$  vs.  $41.42 \pm 3.37$ ). There was no significant difference in total motility between both samples analyzed ( $49.49 \pm 1.31$  vs.  $50.57 \pm 1.41$ ). Concerning progressive motility, a significant difference was found between samples with a decrease of this parameter in samples collected the day of insemination compared with samples collected the day of the initial evaluation ( $86.94 \pm 1.13$  vs.  $71.10 \pm 1.28$ ), in the whole group of patients.

**Conclusions:** The quality of some semen parameters decreases on samples collected the day of IUI, compared to the samples collected at the initial evaluation of male fertility. This detail would be important in the initial male evaluation and selection of assisted reproduction treatment in patients with borderline semen parameters to perform an IUI.

### P-04. Influence of women age, ovarian stimulation protocol and follicular size on the number of Metaphase II oocytes recovered

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**Objective:** This study was performed to investigate how women age, ovarian stimulation protocol and follicular size influence the further number of metaphase two (MII) oocytes recovered in patients treated with intracytoplasmic sperm injection (ICSI).

**Methods:** We analyzed data from 223 cases of ICSI patients, carried out between January 2013 and November 2017. Cases diagnosed with male factor and / or with donor oocytes were included, in which at least four follicles were aspirated and three oocytes recovered with a minimum of two MII oocytes. Poisson regression model was used for statistical analysis, where age of patients and donors, ovarian stimulation protocol, follicular size (measured 36-60 hours before follicular aspiration), number of aspirated follicles, number of recovered oocytes and number of MII-stage oocytes were evaluated to explain the variable number of MII oocytes recovered. The variables were expressed as mean  $\pm$  standard deviation (SD). A *p* value of 0.05 denote significant differences.

**Results:** One hundred forty two cases were included with a patient/donor age range of 18-39 years (Table 1). A significant relationship between the number of MII oocytes recovered and women's age was found, where the average number of MII oocytes decreased by 3.49% each year, either in donors' oocyte or in patients. The effect of ovarian stimulation protocol using GnRH agonists + recombinant FSH (rFSH) had not significant effect on the number of MII oocytes recovered compared to GnRH Antagonists+rFSH. However, a difference was found between GnRH Antagonists+rFSH ( $11.27 \pm 7.15$ ) vs. GnRH Agonists+urinary FSH (uFSH) ( $8.25 \pm 4.58$ ) with an average decrease of 25% of the number of MII oocyte in the last case. Regarding the follicular size, we noted that in cases with 17, 18 or 19 mm of diameter, compared to the cases with measure of 16 mm, the number of MII oocytes increased on average 9.41, 9.71 and 11.22% respectively, decreasing this number when left up to 20 mm size.

**Conclusions:** The increase in maternal age decrease the average number of MII oocytes. Ovarian stimulation with GnRH Antagonist+ FSH increment the MII oocytes recovery, compared with GnRH Agonist + uFSH. The higher number of MII-stage oocytes was achieved when follicular size was from 17 to 19 mm of diameter.

Table 1. Description of the cases				
	Age (years)	Number of aspirated follicles	Number of oocytes recovered	Number of MII-stage oocytes
Mean $\pm$ SD	30.25 $\pm$ 6.04	23.01 $\pm$ 12.62	13.11 $\pm$ 7.74	10.74 $\pm$ 6.94
Ovarian stimulation protocol				Cases (N)
GnRH Antagonist + rFSH (Age: 28.21 $\pm$ 6.24)				58
GnRH Agonist + rFSH (Age: 31.69 $\pm$ 5.56)				72
GnRH Agonist + uFSH (Age: 31.50 $\pm$ 5.37)				12

## P-05. Pursuing the euploid embryo: How many cycles do we need?

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**Objective:** The aim of this study was to determine how many cycles do we need to achieve an euploid blastocyst, grouped by female age and in egg donor cycles.

**Methods:** Patients who underwent preimplantation genetic testing of aneuploidy (PGT-A) cycles (April 2016-March 2018) were considered. PGT-A analysis was performed by using next generation sequencing (NGS) and blastocyst biopsy with their own oocytes categorized by four age groups (<35, 35-37, 38-40, >40 years old) and egg donation. The analyzed variables were: number of MII oocytes retrieved, blastulation rate, number of euploid embryos by ANOVA.

**Results:** A total of 190 cycles were analyzed. The results obtained are shown in table.

**Conclusion:** With the purpose of doing an adequate initial counseling it is important to know our own results and the requirements needed to achieve an euploid blastocyst. Our results demonstrate that, in patients younger than 38 years old, we will probably need only one cycle to achieve an euploid blastocyst. The requirement is considerably higher in patients between the ages 38-42. This information is very valuable for a proper advice. In egg donation cycles, the difference is substantial. This data is very useful for egg donation programs management and has correlation with international research that recommends not doing PGT-A in this cases because of the high euploidy achieved.

Category	Cycles Analysed	Average MII Oocytes	Blastulation rate	Average Euploid Blastocysts
<35	18	13.28	4.5	1.89
35-37	33	9.42	3.27	1.15
38-40	55	8.15	3.09	0.53
>40	44	8.18	2.27	0.29
Egg donor cycle	40	12.9	5.43	2.7

## P-06. Does oocyte age influences morphokinetics score? Oocyte age, Vitrolife's KIDScore and blastulation timing correlations for clinical pregnancy using a Time-lapse system

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**Objective:** Embryo selection is a key-step during IVF treatments. The Time-Lapse system technology has

allowed new options of embryo evaluation based on continuous observation of embryo development. The combination of morphology and kinetics parameters, known as morphokinetics, have emerged as a new tool for embryo selection. Oocyte quality/aging is one of the most determinant factors for IVF success. Our objective is to correlate oocyte age, KIDScore (Known Implantation Data Score, a decision support tool for embryo selection from Vitrolife, Sweden) and time to blastulation with clinical pregnancy.

**Methods:** Twenty-six IVF cycles from January to August 2018, from patients undergoing ART procedures at Huntington Medicina Reprodutiva (São Paulo, Brazil), with known reproductive outcomes after embryo transfer (Single embryo transfer (SET): positive or negative clinical pregnancy and Double embryo transfer (DET): positive twins or negative clinical pregnancy) were selected. All embryos were maintained in the EmbryoScope time-lapse system (Vitrolife) after ICSI procedure until blastocyst (D5/D6). In total, 40 embryos were analyzed. We have included fresh (n=15) and frozen (n=25) embryos and SET (n=12) and DET (n=14). Oocyte age, KIDScore and time to blastulation were analyzed between positive (SET positive, DET double positive) and negative (SET negative; DET double negative) clinical pregnancy outcome. DET with just one gestational sac with heartbeat were excluded from this analysis. Student's T-test and Pearson's correlation coefficient were used as appropriate.

**Results:** Oocytes average age was 33.51(±4.14) years old; oocytes that resulted in embryos with a positive clinical pregnancy outcome (n=17, 42.5%) average age was 32.41 (±3.08) and negative (n=23, 57.5%) was 34.3(±4.60) years old,  $p=0.02$ . KIDScore and time to blastulation were not statistically different between positive and negative clinical pregnancy groups (7.60±1.43 vs. 6.87±2.33,  $p=0.52$  and 102.28±5.65 vs. 104.91±7.16 hours,  $p=0.32$  respectively). The correlation coefficient between these variables showed a weak negative correlation between oocyte age and KIDScore value ( $r= -0.20$ ,  $P=ns$ ) and a strong negative correlation between time to blastulation and KIDScore ( $r= -0.75$ ,  $p<0.0001$ ) for the negative clinical pregnancy group only.

**Conclusion:** Although our data did not show any statistical difference in the time-lapse algorithms to enhance implantation perspective, the potential use of those technologies to predict IVF treatments is a promise for a better embryo selection. The influence of oocyte age on morphokinetics should be explored and may be considered to improve this new powerful tool.

## P-07. Successful delivery of a healthy baby following the transfer of embryos cryopreserved for 17 years

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**Introduction:** Embryo cryopreservation is widely used to provide an additional chance of pregnancy in most cycles of IVF since when Alan Trounson first reported a birth (Trounson & Mohr, 1983). In 2017 a live birth was reported after a transfer of embryo cryopreserved for 24 y in Tennessee - USA. In this poster we report the case of a successful pregnancy from blastocyst-zygote derived after 17y after freezing.

**Case report:** A 29y old female was submitted to infertility treatment as oocyte donor due to severe male factor - oligoasthenozoospermia. The ovarian stimulation cycle was performed with pituitary suppression by GnRH analogue initiated in the second phase of the previous cycle and FSH-r 250 IU/ day. Oocyte retrieval was performed 36 hours after administration of 10,000 IU of hCG (Profasi -Serono). Nineteen oocytes were retrieved and incubated in HTF culture medium. Cumulus cells were removed 2 hours post-retrieval by exposure of 30s hyaluronidase solution 80 IU/mL (Irvine Scientific) and terminated by mechanical pipetting. Seventeen metaphase II oocytes were divided with 8 for the recipient and 9 oocytes were submitted to ICSI microinsemination with sperm of the patient partner according to protocol previously described (Steirteghem *et al.*, 1993). Evaluation of oocytes after 18 hours showed 9 oocytes with normal fertilization (zygotes). All zygotes were frozen due to the risk of hyperstimulation. Freezing was performed by the slow protocol using PROH as cryoprotectant and controlled cooling in 4 steps: Initial temperature: 16°C, -2°C/min to 8°C, -8°C/min for 5 minutes plus manual seeding, -0.3/min to -30°C and -50/min to -150°C, immediate introduction in N2 for storage. Warming was performed after 11 years in decreasing solutions of PROH in the presence of sucrose with 100% survival. The zygotes were cultured in sequential G1/G2 medium (Vitrolife - Gothenburg, Sweden) under mineral oil Ovoil (Vitrolife) at 37°C and 6.0% CO<sub>2</sub> atmosphere. After 120 hours of culture, 4 blastocysts were formed, 2 of which were selected for transfer and 2 for vitrification according to a 2-step protocol (Kuwayama, 2007). The endometrium was prepared with estradiol valerate 6mg/day. When endometrial thickness reached 8 mm the use of micronized progesterone began, and the transfer was carried out 5 days after using Sydney catheter (Cook IVF Australia) monitored by ultrasound. It resulted in single-sac clinical pregnancy that evolved into a miscarriage at 8w. The second transfer was required by the patients after 6 years. On the day of transfer, the 2 blastocysts were heated according to a protocol previously used (Kuwayama, 2007) and cultured for 4 hours in Global Culture medium at 37 °C and 6.5% CO<sub>2</sub> atmosphere. The transfer was performed under ultrasound vision with a catheter by Edwards Wallace (Smiths Medical-UK). The beta-hCG test was positive (122 IU/mL) on day 12 after transfer. A transvaginal ultrasound was performed at 6w, noticing clinical singleton pregnancy. The evolving pregnancy went by without complications and a healthy baby girl was delivered at 39w by c-section in November 2018.

**Comments:** Freezing and warming techniques have evolved since first livebirth from a cryopreserved embryo was described. Today most centers use the technique of vitrification as described by Kuwayama in 2007. The present case shows that even with the slow freezing methods used before we can obtain a favorable outcome with Kuwayama's warming protocol.

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## P-08. Delay in the blastulation day and the aneuploidy rate

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**Objective:** The extended culture of the embryos is a useful tool for the selection of viable embryos, showing that the longer it takes to develop the blastocyst, the chances of implantation and pregnancy are decreasing, so it is defined as day 5 the ideal day of development of the blastocyst, showing pregnancies even on day 7 of embryo culture. Our interest is to show if the highest percentage of aneuploid embryos obtained on day 6 and 7 is due to the late development of the blastocysts or is due to a population of older patients on day 6 and 7.

**Methods:** Results of biopsies of 462 embryos analyzed between 2012 and 2018 are available. Aneuploidy rates were calculated according to the biopsy day and the population was divided according to maternal age (over 37 years). Chi-square association tests were performed with the corresponding Yates's correction when necessary.

**Results:** 59% of the embryos were aneuploidies. The rate of aneuploidy recorded appears to be different according to the day of biopsy (51% on day 5; 61% on Day 6; 68% on Day 7) (Table 1). The chance of aneuploidy is 1.5 times higher in biopsies of day 6 or 7 comparatively with biopsies of day 5 (or = 1,55) (1.3; 2.1)  $p < 0.05$ ). The percentage of aneuploidy for patients older than 37 years was 62% (Table 2). 60% of the embryos analyzed were generated by patients older than 37 years. The proportion of patients older than 37 years whose embryos were biopsied on days 6 and 7 is similar to the whole set of embryos analyzed (62%).

**Conclusions:** The rate of aneuploidy is increased as the time of the biopsy is delayed. The proportion of aneuploid embryos increases directly with maternal age, the analysis of the embryos analyzed on days 6 and 7 shows that there is no difference in the proportion of patients older than 37 years compared to the total studied. Our data show that embryos with delayed blastulation are more likely to be aneuploid, being independent of maternal age.

**Table 1.** Result and biopsy day

Biopsy day	Aneuploid	Euploid	Mosaic	Total
5	72 (51%)	59 (42%)	9 (7%)	140
6	173 (61%)	92 (33%)	76 (6%)	282
7	27 (68%)	9 (23%)	4 (10%)	40
Total	272 (59%)	160 (35%)	30 (6%)	462

**Table 2.** Result and age group

Age group	Aneuploid	Euploid	Mosaic	Total
<37	98 (54%)	70 (38%)	15 (8%)	183
≥37	174 (62%)	90 (32%)	15 (5%)	279
Total	272 (59%)	160 (35%)	30 (6%)	462

## P-09. Ovarian stimulation in cancer patients: Random versus Conventional start

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**Objective:** To determine if random start ovarian stimulation in cancer patients provides similar results compared to conventional stimulation starting in follicular phase.

**Methods:** Retrospective data analysis at a single center (CEGYR). All patients undergoing oocyte cryopreservation for fertility preservation due to recent cancer diagnosis were reviewed from 2012 to 2018. Patients were grouped according to random start or conventional start of the ovarian stimulation. Conventional start was defined as scheduled in early follicular phase initiation of gonadotrophins, random start was initiated at any other moment of the menstrual cycle. The analyzed variables were: number of oocytes, number of matured oocytes (metaphase II), cycle length.

**Results:** 59 cycles met inclusion criteria. Oocytes were collected of 37 (62%) patients on the random start group and 22 (37%) from the conventional one. Mean age was 33.49 years old in the conventional and 32.14 years old in the random start groups. ( $p=0.33$  IC -1.44 to 4.14). The mean number of oocytes collected were similar 12 (conventional) versus 11.09 (random) ( $p=0.60$  IC 3.04 to 5.18) and mean number of mature oocytes vitrified was also similar (metaphase II): 9.70 (conventional) vs. 8.04 (random) ( $p=0.36$  IC 1.97 to 5.28). The cycle duration was different, being the conventional shorter (9.21 days) than the random group (11.05 days) ( $p=0.01$  IC 0.24 to 2.17).

**Conclusion:** Random start stimulation cycles for cancer patients has comparable results and allows patients to start gonadotrophin stimulation irrespective of menstrual cycle phase, with no impairment of oocyte yield and only a small increase of cycle duration. Random start is a good opportunity for patients who are run out of time and face a fertility threatening medical condition.

## P-10. Argentinian experience of preimplantation genetic testing for chromosomal structural rearrangements using Microarray-based Comparative Genomic Hybridization and Next-Generation Sequencing

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**Objective:** The incidence of balanced translocations was determined to be 1 in 500 in the general population. However, this incidence is substantially higher in infertile patients. Reciprocal and Robertsonian translocations carriers have an increased risk of producing unbalanced gametes which often cause spontaneous miscarriage, children with congenital anomalies or intellectual disability.

The aim of this study was to share our experience doing preimplantation genetic testing for chromosomal structural rearrangements (PGT-SR) by Microarray-based Comparative Genomic Hybridization (aCGH) and Next-Generation Sequencing (NGS).

**Methods:** This study included all patients who underwent PGT-SR (18 reciprocal translocation cases, 9 Robertsonian translocations cases and 1 insertion case, Table 1) between 2013 and 2018 at a single fertility center. There were 154 trophoctoderm biopsies, contributed by 28 patients, with a median age of 31.8 years (20 - 43 y/o). All samples were subjected to whole genome amplification (WGA) by the SurePlex (Illumina®). aCGH analysis was carried out using the 24Sure Cytochip (BlueGnome-Illumina®) (between 2013 - 2016) and NGS analysis was performed by the VeriSeq MiSeq (Illumina®) (from 2016 to 2018), both according to the manufacturer's protocol (Illumina, UK). Results were analyzed by BlueFuse Multi software (Illumina, UK).

**Results:** For each couple, an average of 4.8 (1-14) blastocysts was obtained, in one or more controlled ovarian hyperstimulation cycles. 22 couples underwent a single cycle, while 6 couples needed two cycles. A total of 150 blastocysts were finally studied (DNA from 4 trophoctoderm biopsies, 2.6%, did not amplify). The results are described in Table 2, except for two samples with degraded DNA result. Each couple obtained on average 1.3 (0-5) blastocysts to transfer (balanced for the chromosomal rearrangement and euploid). During the period covered, 28 embryo transfers were performed after thawing, resulting in 13 (50%) positive HCG-beta subunit tests. The implantation rate was 42.9% (12) and the abortion rate, 16.7% (2).

**Conclusions:** PGT-SR in couples with one member carrying a chromosomal rearrangement allows the transfer of a balanced and euploid embryo, which reduces the risk of miscarriage and affected newborn. According to our results, only 23.6% of the blastocysts studied are transferable, which highlights the importance of carrying out embryo testing in these cases.

Table 1. Chromosome rearrangements that warranted PGT-SR cycles				
	Chromosomal rearrangement	Total nº of couples	Chromosomal rearrangement	Total nº of couples
Reciprocal translocations	46, XY, t(8;9) (q10;p10)	1	46, t(3;10) (p25;q11.2)	1
	46, XY, t(6;20) (p21.3;q11.2)	1	46, XY, t(9;18) (q22;p11)	1
	46, XY, t(3;15) (p12;q14)	1	46, XX, t(10;18) (q11.2;q11.2)	1
	46, XY, t(5;15) (q35.1;q22.1)	1	46, XX, t(3;9) (q12;q13)	1
	46, XY, t(10;13) (q21.3;q21.2)	1	46, XY, t(2;12) (p13;q22)	1
	46, XY, t(2;5) (q21;p13)	1	46, XY, t(15;17) (q15;p13)	1
	46, XY, t(1;22) (p34;p13)	1	46, XY, t(1;14) (q25.3;q31)	1
	46, XY, t(10;13) (q21.3;q21.2)	1	46, XX, t(4;13) (p10;q10)	1
46, XX, t(2;4) (p12;p14)	1	46, XY, t(7;10) (q11.2;q22)	1	
Robertsonian translocations	45, XX, t(13;14) (q10;q10)	2	45, XY, t(13;14) (q10;q10)	7
Insertion	46, XY; ins(7;8)(q35q36.3;p23.3)			1

**Table 2.** Embryo chromosome results of the PGT-SR cycles

Status for paternal translocation	Total	Euploidy	Aneuploidy	Mosaic
Balanced	66 (44.6%)	35 (23.6%)	21 (14.2%)	10 (6.8%)
Unbalanced	82 (55.4%)	49 (33.1%)	28 (18.9%)	5 (3.4%)

## P-11. Results from more than 1000 embryos analyzed in-house for preimplantation genetic testing for aneuploidies

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**Objective:** The goal of preimplantation genetic testing for aneuploidy (PGT-A) is to select euploid embryos for transfer in order to improve the probability of conceiving a viable pregnancy. In this way, the risk of miscarriage and complications related to an affected pregnancy decreases. The objective of this study was to describe our experience in more than 1000 embryos analyzed for PGT-A according to maternal age and PGT-A indication.

**Methods:** A total of 410 PGT-A cycles were performed in a single fertility center between April 2013 and December 2018. Embryos were cultured up to day 5 or 6. Trophoctoderm cells were biopsied and then blastocysts were vitrified. The DNA from 1378 trophoctoderm biopsies was amplified (SurePlex, Illumina®) in our genetic laboratory. Chromosomal evaluation was carried out by comparative genomic hybridization array (24Sure Cytochip, BlueGnome-Illumina®) for 382 embryos from 2013 to 2016, and by massive parallel sequencing (NGS) (VeriSeq-PGS, Illumina®) for 970 embryos since 2016. Results were analyzed according to maternal age ( $\leq 34$ , 35-37, 38-40 and  $>40$  years old) and PGT-A indications: time to pregnancy, recurrent pregnancy loss (RPL) ( $\geq 2$  miscarriages), repeated IVF failures, advanced maternal age (AMA) ( $\geq 38$  years old). Euploid embryos were devitrified and transferred. Clinical pregnancy rate and miscarriage rate were evaluated. For statistical analysis, we applied the Chi-Squared test.

**Results:** From the 1378 embryo biopsies, 1.9% (26) have no DNA amplification. A total of 1352 blastocysts were finally studied. Euploidy rate was 54.3% for aCGH results. Since the application of the NGS methodology, embryo mosaicism was able to be detected (23.8%) and euploidy rate was 40.8%. Considering only NGS results, euploidy rate was 58.0% in women  $\leq 34$  years old, decreasing to 36.2% for women between 35-37, 27.6% between 38-40 years old and 15.6% for women more than 40 years old ( $P$ -value  $< 0.05$ ). Results according to PGT-A indication (studied by both methodologies) are described in Table 1. As expected, euploidy rate was lower in AMA cycles and AMA combined with RPL. In these groups, more than half of the cycles have no euploid embryos. The clinical pregnancy rate for AMA was similar to cycles with time to pregnancy indication and RPL. However, repeated IVF failure cycles showed the less clinical pregnancy rate, being the group less benefited from PGT-A. Low miscarriages rates also highlight the advantage of PGT-A, although repeated IVF failure and RPL + AMA seems not to improve.

**Conclusions:** The results of our PGT-A program generated useful data for reproductive counseling. It is important to mention that more than half of AMA patients will have no normal embryo for transfer. However, when an euploid embryo is transferred, pregnancy rates are comparable to younger women, decreasing the risk of miscarriages. For RPL indication, PGT-A also resulted in an appropriate choice for reducing the risk of another miscarriage.

**Table 1.** Results from the different PGT-A indications

	Time to pregnancy	Recurrent pregnancy loss	Repeated IVF failure	Advanced maternal age	RPL + AMA
Cycles	132	41	41	181	15
Age rate	28.7 $\pm$ 5.4	35.3 $\pm$ 2.4	35.6 $\pm$ 2.7	40.9 $\pm$ 1.9	40 1 $\pm$ 1.3
Euploidy rate	58.4%	50.7%	45.1%	27.1%	21.1%
Cycles with no euploid embryo	8.3% (11)	22% (9)	22% (9)	55.2 (100)	66.7% (10)
Number of transfers	124	33	39	48	21
Clinical pregnancy/transfer	56.5%	48.5%	38.5%	45.8%	42.9%
Miscarriage	5.1%	0%	18.8%	4.2%	30.0%

## P-12. Evaluation of oocitary dysmorphism under seasons influence

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**Objective:** The oocyte quality is extremely relevant to Assisted Reproductive Technology (ART). In the search of better rates, it is important to understand all factors that influence in oocyte quality. In this manner, the objective of this study was to evaluate the influence of seasons in oocyte quality of 71 patients. All patients were undergone ART to infertility treatment at Androlab, an human reproduction clinic in Curitiba/PR (Brazil).

**Methods:** Patients were separated into two groups according to the season when ovarian stimulation occurred. Summer group was composed of patients who were submitted to ovarian stimulation in a period of 21/12/2016 to 20/03/2017. Winter group was patients that were submitted to ovarian stimulation in a period of 21/06/2017 to 20/09/2017. All collected oocytes were analysed and classified according to oocitary dysmorphisms: zona pelúcida thickness, large perivitelline space, granules in the perivitelline space, oocyte shape, oocyte color, oocyte size, fragmented polar body, polar body shape, polar body size, vacuoles, refractile bodies, gelatinous aspect, dark coarse granules and granules in the cytoplasm.

**Results:** A total of 605 oocytes were evaluated from 71 patients. Summer group had 197 oocytes and winter group had 408 oocytes. The alterations that showed statistically significant differences ( $p < 0.001$ ) were: polar body shape, presence of refractile bodies, gelatinous cytoplasm and cytoplasmic granulation.

**Conclusion:** Adverse environmental conditions, such as temperature variations, can cause an increase or decrease in internal body temperature, resulting in thermal stress. This stress may affect oocyte quality, leading to oocyte dysmorphisms. Studies have shown that extracytoplasmic abnormalities do not interfere in embryonic development. In contrast, cytoplasmic changes may indicate cytoplasmic immaturity and interfere with the rate of fertilization and embryonic development. Cytoplasmic dysmorphisms presented greater difference in relation to the seasons of the year. Higher incidence of granulation and presence of refractile bodies were observed mainly in the winter period. In conclusion, the season of the year interferes in the oocyte quality, mainly the cytoplasmic quality of the oocyte.

	Summer 2017		Winter 2017		p
	Count	%	Count	%	
Pellucide Zone Thickness	144	73%	249	61%	0.04
Perivitelline Space Increased	37	19%	113	28%	0.017
Granules in the Perivitelline Space	14	7%	64	16%	0.03
Oocyte Shape	14	7%	27	7%	0.823
Oocyte Color	18	9%	72	18%	0.006
Oocyte Size	3	2%	2	0%	0.189
Fragmented Pollar Body	60	30%	168	41%	0.11
Polar Body Size	1	1%	11	3%	0.07
Polar Body Shape	0	0	51	13%	<0.001

	Summer 2017		Winter 2017		p
	Count	%	Count	%	
Vacuoles	9	5%	7	2%	0.04
Reticulum	0	0%	7	2%	0.064
Refractile Bodies	14	7%	237	58%	<0.001
Gelatinous Cytoplasm	0	0%	46	11%	<0.001
Granules in the Cytoplasm	13	7%	172	42%	<0.001
Dark Coarse Granules	42	21%	48	12%	0.002

### P-13. correlation between oocitary dysmorphism and the patient age

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**Objective:** The advancement of age is considered a determining factor in female fertility. As age increases, especially after age 40, there is a decrease in ovarian reserve and loss of oocyte quality. Variations in oocyte morphology may occur due to factors such as age, genetic problems and environmental changes. Thus, the objective of the present study was to evaluate the age of the woman as an

interfering factor in oocyte quality of patients undergone infertility treatment from a human reproduction clinic in Curitiba / PR (Brazil).

**Methods:** The patients were divided into 3 groups according to the age: Group 1 - less than or equal to 35 years; Group 2 - between 36 and 39 years and Group 3 - greater than or equal to 40 years. It was evaluated the oocitary dysmorphisms: large pellucide zone, perivitelline space increased, granules in the perivitelline space, oocyte shape, oocyte color, oocyte size, fragmented polar body, polar body size, polar body shape, vacuoles, reticulum, refractile bodies, gelatinous cytoplasmic, granules in the cytoplasm and dark central granules.

**Results:** It was analysed a total of 1947 oocytes. 1418 oocytes were from patients aged 35 years or less, 431 oocytes from patients aged 35-40 years and 98 from patients aged 40 years or older. There were significant statistical differences for the presence of granulation in the cytoplasm, refractile bodies and gelatinous cytoplasm ( $p < 0.001$ ). Patients in group 3 had 98% of the oocytes with refractile bodies, while the patients in the other groups had only 50% of the oocytes with this dysmorphism. For the presence of granulation, the patients in group 3 obtained 85% of the oocytes with the alteration, while the patients in group 1 and group 2 had 50% and 51% of the oocytes with granulation, respectively. The gelatinous cytoplasm was observed in group 3 in 26% of the oocytes, in group 1 in 12% and in group 2 in 16% of the oocytes analyzed. The other alterations did not show a significant difference between the groups

**Conclusion:** The advancement of age may influence the formation of oocyte alterations. An oocyte with normal and mature morphology presents a rounded form, presence of an intact polar corpuscle, clear pellucid zone, small perivitelline space, transparent and homogeneous cytoplasm and absence of granulations. Oocyte alterations can be divided into two types: cytoplasmic and extra-cytoplasmic. Studies have shown that extra-cytoplasm does not interfere with embryonic development. Cytoplasmic alterations, however, interfere with the rate of fertilization and embryonic development. Cytoplasmic changes may indicate cytoplasmic immaturity, which may be caused by meiotic spindle anomalies, which are more common in women over 40 years of age. Thus, the conclusion of the present study corroborates with information from previous publications that 40 years is the determining age in the reproductive life of women.

### P-14. Standard of seminal analysis in patients with normal and altered BMI from an assisted reproduction clinic

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**Objective:** Numerous scientific studies indicate that obesity and overweight can affect seminal quality through endocrine or molecular changes. The latest Latin American data released by the World Health Organization (WHO) showed that obesity in Central and South America reached 25-30% of the population. The objective of this study was to analyze the main seminal characteristics (volume, concentration, motility and morphology) of 738 patients who search for an Assisted Reproduction Clinic (ANDROLAB) in

Curitiba, Brazil, to perform a spermogram evaluation test and relate it to their Body Mass Index (BMI).

**Methods:** The data collection was performed through a questionnaire applied to the patients who sought the private laboratory to perform sperm analysis. The BMI was calculated from the data provided in the questionnaire with the formula  $BMI = \text{weight (kg)}/\text{height (m}^2\text{)}$ , being considered with adequate weight those patients whose BMI were greater or equal to 18.5 and less than 25; with overweight those with BMI greater than or equal to 25 and less than 30 and obese with BMI greater than or equal to 30. This classification follows the recommendation of the Brazilian Health Ministry. To make easier the visualization of the results the patients were separated into two groups: those with BMI higher than 25 here named "high BMI" (n=468) and those with BMI below 24.9 referred as "adequate BMI" (n=265). 1% of the patients (n=5) had a BMI lower than 18.5, being considered undernourished and, therefore, were excluded from the analyzed groups. The seminal analysis was performed according to WHO standards in its guide Manual for the examination and processing of human semen of 2010. Values considered normal were: seminal volume  $\geq 1.5$  mL, concentration  $\geq 15$  million sperm per mL, motility  $\geq 32\%$  progressive moving spermatozoa and morphology at least 4% normal spermatozoa (Kruger standard).

**Results:** The mean values found were: 3.8mL (0.1mL-12.5mL), concentration 64 million spermatozoa per mL (0-540), motility 37% progressive spermatozoa (0%-85%) and morphology 4% of normal sperm (0-9%). Regarding weight, 63% of the patients presented high BMI. In the group "adequate BMI" the incidence of alterations were: 3% of volume changes; 14% of concentration; 32% motility and 42% morphology. Whereas in the group "BMI High" the changes were: 7% of volume; 22% of concentration; 37% motility and 47% morphology. There was a statistical difference in volume ( $p=0.05$ ) and concentration ( $p=0.02$ ). Motility ( $p=0.199$ ) and morphology ( $p=0.181$ ) showed no statistical change.

**Conclusion:** Obesity is a morbidity whose incidence has grown worldwide. In our study, seminal volume and concentration were significantly lower in patients with high BMI than in those with normal BMI. This data is similar to other studies in the area of obesity and male infertility, which suggest a decrease in testosterone levels and an increase in estradiol levels as the main cause of these changes in overweight or obese men.

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## P-15. Comparison of three expanded carrier screening panels at a single fertility centre

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**Objective:** It is estimated that 1-3% of couples are at risk of having a child affected by a recessive disease. The main purpose of preconception carrier tests is to identify these couples, thereby enabling consideration of alternative reproduction options and early intervention strategies in order to reduce the risk of occurrence of the disorder. The aim of the present study was to compare three different expanded carrier screening (ECS) tests for recessive disorders.

**Methods:** A total of 687 patients who underwent ECS from 3 different laboratories (Panel A, Panel B and Panel C), between 2014 and 2018, were included. A consent form was signed by each individual. All patients received genetic counseling before and after the test. For Panel A and C, blood samples were taken and DNA was extracted and sent to external laboratories. For Panel B, blood samples were collected, processed, sequenced and analyzed in our laboratory. Panel A was performed by SNP array genotyping. Panel B was performed using full-exon sequencing by Next Generation Sequencing (NGS), a method that can identify all single nucleotide variants and small insertions and deletions in exons and intron-exon boundaries. Finally, Panel C used "targeted genotyping" (TG) of a set of predefined pathogenic variants by NGS. Additional complementary techniques dPCR and TP-PCR, MLPA and TP-PCR, were carried out in Panel A and B respectively, in order to study Spinal Muscular Atrophy and Fragile X Syndrome. For panel C, only TP-PCR was used. Panel content, carrier rates, carrier couple rates and the 10 most prevalent genetic disorders were compared between panels.

**Results:** Panel A was composed of 302 genes (314 autosomal and X-linked recessive disorders), Panel B covered 484 genes (688 disorders) and Panel C had 299 genes (363 disorders). 112 genes were shared by the three panels. Considering the 412 patients studied with Panel A, 160 (38.8%) resulted to be carriers for 59 different diseases. From the 136 patients screened with Panel B, 112 were identified as carriers (82.3%) for 93 diseases. Panel C identified 81/139 as carriers (58.3%), for 63 different disorders. Regarding couples with mutations in the same gene, Panel B identified 4 out of 22 couples studied, and Panel C 1 out of 5. In Panel A only egg donors were studied. There were two couples screened by Panel B that would not have been identified as carriers of the same disorder by panel C because in one case the gene (*WNT10A*) was not included and neither was the variant (c.601G>A, *CFTR*) in the other case. The 10 most prevalent genetic diseases detected in the three panels are shown in Table 1.

**Conclusions:** In this study, we compare three ECS panels each with different methodological approaches which had its advantages and disadvantages. Our data showed that carrier rates vary widely based on panel content and type of analysis. Panel B would potentially detect all pathogenic variants in the coding sequence of the genes included. However, the analysis and interpretation of the results is challenging and time-consuming. Panel A and C study a pre-selected group of clear pathogenic variants, thus, the analysis is simple and fast, but several mutations will not be detected. This assumption may be confirmed by the finding that Panel B identified a higher proportion of carriers, because of the methodology used and the number of genes covered. When comparing ECS panels, it is important to understand exactly their differences, similarities and limitations for the appropriate genetic counseling of each individual/couple. Our results highlight the need to incorporate ECS coupled with genetic counselling into routine assisted reproduction practice.

**Table 1.** Comparison of carrier frequencies for top 10 disorders observed in each panel

Panel A		Panel B		Panel C	
Disorder	Carriers	Disorder	Carriers	Disorder	Carriers
GJB2-Related Nonsyndromic hearing loss and deafness	26	Familial Mediterranean Fever	14	Alpha-1 antitrypsin deficiency	14
Biotinidase Deficiency	19	CFTR-Related disorders	14	21-hydroxylase deficiency	11
Cystic Fibrosis	11	Wilson disease	14	Alpha thalassemia	8
Familial Mediterranean Fever	10	GJB2-Related Nonsyndromic hearing loss and deafness	7	CFTR-Related disorders	7
Pseudocholinesterase Deficiency	10	SMN1 Linked Spinal Muscular Atrophy	7	Familial Mediterranean Fever	7
SMN1 Linked Spinal Muscular Atrophy	8	Progressive familial intrahepatic cholestasis	7	Tyrosinemia type I	7
Hereditary Fructose Intolerance	6	Primary coenzyme Q10 deficiency	7	Oculocutaneous albinism type II	5
Type I Glutaric Acidemia	6	Muscular dystrophy-dystroglycanopathy type C3	7	SMN1 Linked Spinal Muscular Atrophy	5
Classical Galactosemia	5	Cystic Fibrosis	6	Glucose-6-phosphate dehydrogenase deficiency	5
Glycogen Storage Disease: Type II	5	Classical Galactosemia	6	GJB2-Related Nonsyndromic hearing loss and deafness	4

## P-16. Analysis of products of conception by next-generation sequencing

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**Objective:** The major cause of first trimester miscarriages is chromosomal aneuploidies (50-70% of cases). Conventional cytogenetic analysis is the traditional method for the chromosomal testing of the products of conception (POC). The benefits are determined by limitations of the G-banded karyotyping on cultured cells method and risk of maternal cell contamination (MCC). Molecular karyotyping by next-generation sequencing overcomes the limitations of other techniques because it does not require cell culture and allows all chromosomes detection. Here we describe our experience using next-generation sequencing (NGS) for product of conception studies.

**Methods:** 46 POCs and maternal blood samples were referred to our laboratory between November 2016 and December 2018. POCs were obtained from spontaneous abortions (14), curettage/aspiration (15) or hysteroscopy/embryoscopy (17). Following DNA extraction, POC samples were analyzed by NGS (VeriSeq-Illumina). Normal and female samples (karyotype 46, XX) were fingerprinted by STRs for 16 markers to detect maternal cell contamination (MCC) and XXX triploidy.

**Results:** The average maternal age was 36.7 (29-44y/o) and the average week of developmental arrest was 8 (5.1-18 weeks). Among 46 fetal miscarriage samples, 3 (6.5%) were inconclusive due to MCC. Concerning samples studied, 12 POCs (26.1%) were euploid. Two-thirds of samples studied (31, 67.4%) carried at least one chromosomal abnormality and a subset (25) was identified as autosomal trisomy which accounts for 80.6% of aneuploidies samples. This group included

two cases with double trisomy (involving chromosome 15 and 21, 16 and 21) and another case with low-grade mosaicism for trisomy 10 (20-40%) in combination with high-grade mosaicism for monosomy X (40-80%). So far, trisomy 16 was the most prevalent (6 cases, 24% of trisomies). Regarding monosomies, there were two cases with Turner syndrome, three cases with high-grade mosaicism for monosomy X and one case with low-grade mosaicism for monosomy 18. The aneuploidy rate in women  $\leq 35$  years was 32.3% (10) and  $>35$  years was 67.7% (21).

**Conclusions:** Analysis of products of conception using next-generation sequencing was a faster method than the conventional karyotype and has a low no-result rate. In our experience, we obtained results in 93.5% of the cases. In 3 samples MCC was confirmed. This study could be carried out with miscarriages from pregnancies achieved by natural conception or by assisted reproductive technology interventions, especially in couples with recurrent pregnancy loss. POC analysis is essential to determine the cause of pregnancy loss. This information is relevant to estimate the risk of recurrence, to avoid unnecessary further studies and to plan new reproductive strategies.

## P-17. Comparison of CFTR mutation frequency using three different expanded carrier screening tests in an egg donation program

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Soledad Sepulveda Award

Best Poster Presentation

14<sup>th</sup> REDLARA General Congress, Merida, Mexico - 2019

**Objective:** Oocyte donation is an integral part of modern assisted reproductive technology (ART). Gamete donors routinely undergo genetic screening testing in order to maximize the health of donor-conceived offspring. Cystic fibrosis (CF) is one of the most frequent autosomal recessive diseases in the Caucasian population, with a general incidence of 1 in 2500 births (1/25 carrier rate). In the Autonomous City of Buenos Aires, the incidence has been estimated in 1 in 6100 births (1/40 carriers). For general-population CF carrier screening, the American College of Medical Genetics and Genomics/American College of Obstetricians and Gynecologists have recommended a core panel of 23 mutations that identify 49-98% of carriers, depending on ethnic background. However, to date, 1828 *CFTR* gene mutations have been identified as pathogenic or likely pathogenic (Professional Human Gene Mutation Database). The objective of this study was to determine the frequency of carriers for classic CF according to three different expanded carrier screening (ECS) tests used in an egg donation program.

**Methods:** A total of 530 oocytes donors candidates (21-33 years old) were included during 2014-2018. All donors signed an informed consent form and were evaluated by a clinical geneticist in order to detect relevant family history. The egg donation program used three different ECS panels for autosomal and X-linked recessive diseases. 412 candidates were assessed with SNP array genotyping (Panel A), 74 by targeted genotyping using Next-Generation Sequencing (NGS) (Panel B) and 44 with full-exon NGS sequencing (Panel C). Panel A tests for 150 *CFTR* gene mutations, panel B analyzes 412 variants, and panel C can identify all single nucleotide variants and small insertions and deletions, in the exons and intron-exon boundaries of the *CFTR* gene.

**Results:** Using Panel A, CF carrier frequency resulted in 1/37. Regarding Panel B and C, the amount of donors studied was small to estimate a representative carrier frequency rate. However, considering the 74 donors studied, Panel B identified 2 as CF carriers and Panel C detected 5 CF carriers out of 44 donor candidates. Among the three panels, 135 mutations were shared. If only the 135 mutations shared between the three panels were considered, the overall CF carrier frequency would be 1/41.

**Conclusions:** Full-exon NGS-based ECS will likely reveal a higher carrier rate than previously reported, as our preliminary results of Panel C showed. In addition, *CFTR* is responsible for phenotypes milder than CF, such as recurrent pancreatitis, bronchiectasis and congenital bilateral absence of the vas deferens, and full-exon sequencing is able to detect variants associated with these conditions as well. The carrier frequency of *CFTR* mutations in the general population has not been estimated in our country, but only derived indirectly from the international prevalence of the disorder. The carrier rate of 1/41 could be used as an approximation of such frequency in our population. This study highlights the importance of applying ECS panels in an egg donation program which could potentially help to reduce the incidence of CF in the offspring of patients undergoing ART.

## P-18. Is the oocyte fertilization affected by maternal age?: An analysis in 47,454 inseminated oocytes

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**Objective:** To evaluate if fertilization process is affected by maternal age and insemination technique.

**Methods:** We retrospectively analyzed 6746 cycles which were performed from January 2004 to December 2018. Cycles were included and classified according to maternal age and insemination technique (either ICSI or FIV). The exclusions criteria were FIV+ICSI cycles, oocyte in vitro maturation, oocyte donor cycles, ICSI with cryopreserved oocytes, severe male factor and patients with diagnosis of pathology or genetic diseases.

**Results:** In the present study, 47 454 oocytes were inseminated either by FIV or ICSI, the presence of two pronucleus were considered as a normal fertilized oocyte. During the period of the study, the preferred insemination technique used was ICSI (51.5%). The fertilization rate was 72% in ICSI vs 68% in FIV ( $p < 0.001$ ). When insemination technique was stratified by age results were shown in Table 1. Additionally, an intra-analysis according to the insemination technique demonstrated that fertilization rate was similar between age groups in FIV ( $p = 0.187$ ) and ICSI cycles ( $p = 0.971$ ). **Conclusions:** Two conclusions can be raise from our study. Firstly, the fertilization rate was similar among maternal age. These results suggest that gamete interaction; oocyte activation and sperm nuclear chromatin decondensation during fertilization process could not be affected by maternal age. Nevertheless, it is well documented that developmental competence and ploidy rate is affected. Secondly, the significantly higher fertilization rate in ICSI should be considered with caution because in FIV cycles all retrieves oocytes have been inseminated while in ICSI only matured oocytes have been considered for injection. Therefore, both insemination techniques have the same fertilization yield.

Table 1. Results.

Age SART Classification (years)	Insemination Technique				p-value
	FIV		ICSI		
	Number of cycles	Fertilization Rate	Number of cycles	Fertilization Rate	
Lower than 35	962	69% (6438/9389)	1027	71% (6324/8848)	<0.001
35-37	877	67% (4507/6715)	919	73% (4629/6373)	<0.001
38-40	874	68% (3721/5453)	940	72% (3931/5430)	<0.001
41-42	378	65% (1284/1981)	348	71% (1169/1636)	<0.001
Higher than 42	178	70% (472/679)	243	73% (691/950)	0.1727

## P-19. Associated factors with total fertilization failure after assisted reproductive treatments: Analysis of 14 years

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**Objective:** To determine which are the associated factor related to total fertilization failure (TFF).

**Methods:** This retrospective cohort study analyses autologous cancelled cycles performed in PRANOR, Assisted reproduction laboratory, Lima - Peru from January 2004 to December 2018. The following parameters were evaluated: type of insemination (FIV or ICSI), maternal and paternal age, physicians, embryologist who performed the insemination, number of inseminated oocytes and seminal parameters. Oocyte and semen donor cycles and severe male factor were excluded from the analysis.

**Results:** A total of 7858 cycles were performed with autologous gametes from January 2004 to December 2018. In total, 1885 cycles (24%) were cancelled. 201 cycles were cancelled because non-oocyte was retrieved (10.6%). From the remaining 1684 cycles, 22.2% (374 cycles) were TFF and 77.8% (1310 cycles) were cancelled due to non-embryo development (NED). Despite women age was similar between TFF (38.3±4.1) and NED (38.4±4.2) ( $p=0.915$ ). The fertilization failure rate was significant lower in ICSI (18.5% (173/933)) vs. FIV (26.7% (201/751));  $p<0.001$ . These results were also obtained when maternal age was stratified following SART classification. Finally, a logistic regression analysis was performed to identify which parameters are independently associated to TFF. Our analysis showed that the number of inseminated oocytes, type of insemination, maternal age and embryologist are related to TFF.

**Conclusions:** Our data suggest that TFF could be explained by multiple factors; some are intrinsic to patient's characteristics and others related to assisted reproductive procedures.

## P-20. Report of pregnancy success after in vitro fertilization in women aged 40 and over with fresh non donor oocytes in Red Crea, Mexico

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**Objective:** The aim of this study was to evaluate the outcomes in women over 40 years in fresh non donor in vitro fertilization (IVF), and compare pregnancy rates with blastocyst transfer (BT), day 2 and day 3 embryo transfer (ET).

**Methods:** This retrospective study was performed among women  $\geq 40$  years old that used their own eggs for IVF between 2013 and 2018 in Red Crea Medicina Reproductiva, Mexico. The study group included 191 IVF cycles in women ages 40 to 47 years who had from 1 to 3 embryos on day 2, 3 or 5.

**Results:** The mean age of the women was 41 years, with 52.8% under 41, 30.8% between 42 and 43, and 16.1% over 44 years. Of the 191 cycles that were included, ET performed in 142 (74.3%), the mean number of ET was 2.1. The general clinical pregnancy rate was 18.9% and 15.6% of live birth rate. Only in 5 cycles, blastocyst transfer was performed, in 82 cycles on day 3 ET was performed and in 54 cycles ET was in day 2. The pregnancy and live birth rate was similar in the blastocyst transfer group and the day 2 and 3 ET group. The clinical pregnancy and live birth rate in BT was 20.0%, in day 2 ET was 11.1% and 7.4% respectively and in day 3 ET the rates were 25.6% and 19.5% respectively. The live birth rate was increased a little with BT (20%) compared with day 2 (7.4%) and 3 (19.5%) ET. The BT group had almost the same embryos transferred per patient compared with the day 2 and 3 ET group.

**Conclusion:** IVF has acceptable outcomes in women 40 and 41 years of age when patient's own oocytes are used. Our data support that women 45 years and beyond do not benefit from IVF procedures using their own oocytes. The day 3 ET group in our study had higher pregnancy rate compared with BT group, but BT appears to be as effective as day 3 ET in older women. It is important to include more BT cases in older women to improve the analysis of this study.

## P-21. Case report of severe male factor in old age and Preimplantation Genetic Diagnosis

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**Case report:** Advanced paternal age, morphology and low sperm concentration have been associated with an increased risk of chromosomal alterations due to sperm DNA damage, telomeric shortening and senescence or apoptosis leading to accumulations of Novo for genetic diseases, recent studies report higher rate of global aneuploidy increasing trisomies 21, 18 or 13 in men over 50 years of age. We report a unique case of a 54-year-old man with a history of severe oligoteratozoospermia and no history of chronic degenerative diseases, evidence of a normal male phenotype and highly complex fertility treatment. Six cycles were performed with donors  $\leq 30$  years, normal karyotype, without chronic degenerative history. In the cycles performed, the same medication was used for ovarian stimulation and similar sperm parameters were reported in the patient. The analyzes report mostly aneuploidies, an increase in monosomy X, a low percentage in the appearance of trisomies. It has been shown that in women over 35 years of age chromosomal mutations increase in this case the donors were in a younger age and with good oocyte quality, so the sperm quality directly affected the embryo quality.

## P-22. PGS contribution to pregnancy rates in different age groups, with own eggs

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**Objective:** This study aims to compare pregnancy rates in 3 age groups (own eggs cycles) when using PGS (preimplantation genetic screening) compared to not using PGS. All the patients were in a program of freeze- all embryos, with a time-lapse system culture with a tri-gas incubator.

**Methods:** 37 embryo transfer cycles with frozen embryo transfer, divided into three age groups:  $\leq 35$ , 36-38 and  $\leq 39$ , between May/2017 and August/2018 were analyzed. All embryos were cultured in an ESCO-MIRI time-lapse system and vitrified/thawed with Cryotech method.

**Results:** There was a general difference in clinical pregnancy rates between the PGS and non-PGS groups (55% vs. 29.4%, respectively). The pregnancy rate in patients younger than 35 years old was 22.2% with non-tested embryos vs. 50% with normal embryos. In patients between 36 and 38 years old, we observed 50% pregnancy rate with non-tested embryos vs. 55.6% with normal embryos. In patients older than 39 years old we observed 25% when non-transferred embryos were transferred compared to 57.1% with normal embryos.

**Conclusion:** In our study we observed a general increase of pregnancy rate when we transferred normal embryos compared with the non-tested embryos transfers. The goal of every assisted treatment is to select the most competent embryo in order to achieve a pregnancy and therefore a healthy new born. Time-lapse culture and PGS are useful methods used to help in this task. Time-lapse offers the opportunity to observe the embryos development without any disturbances, avoiding unnecessary environmental stressors. PGS is used to select the embryos without chromosomal abnormalities. So, performing both methods (time-lapse + PGS) can improve the clinical outcome in own eggs cycles.

## P-23. Have we reached the peak in vitrification? Improved vitrification method yields to the highest worldwide positive pregnancy outcomes compared to fresh cycles

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**Objective:** Many techniques for preservation of oocytes and embryos have been developed during the past 2 decades due to the increasing demand of fertility preservation, either because of health conditions or maternity delay. For this reason, vast clinical results exceed 2,000,000 cases in over 73 countries, in which the open system of

vitrification has been able to make 90% of human oocytes and embryos survive after freezing. Despite the variety of techniques, many of them require very skilled manipulation and complicated protocol; besides, none of them had reached a high survival rate that would consider the fragility of the oocytes of cancer patients or women over 40 years old. A remaining challenge was to rescue valuable oocytes and embryos that still had led to death by creating an improved noninvasive vitrification method that gives possibilities to women in true difficulty and pain. The aim of this work is to present the world-wide results of a very successful vitrification method for oocytes and blastocysts.

**Methods:** In this retrospective study we report patients undergoing IVF cycles during 2015-2017 with either oocyte or blastocyst vitrification in clinics worldwide. We show the hidden improvements of the vitrification method in order to obtain the best survival rates for oocytes and blastocysts. We also compare the pregnancy rates of the cycles with vitrified oocytes and blastocysts with the rates obtained from fresh cycles.

**Results:** The modifications featured in this new technique yield to a worldwide higher pregnancy rate (47% and 48%;  $n=4,673$  and  $n=15,177$  in oocytes and blastocysts respectively) compared to fresh cycles (39%;  $n=115,610$ ; SART, 2016).

**Conclusions:** These results are an evidence of the safety and effectiveness of this improved method. With this high survival rate and excellent clinical results, ordinary methods are changing in to advanced fertility facilities all around the world.

## P-24. Occupational Risk - induced sperm DNA damage assessed by comet assay: a pilot study

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**Objective:** The male factor contributes up to 50% in the problems of infertility in couples. Currently, there are reports of a reduction in male fertility caused by both occupational and environmental exposures that affect sperm DNA stability. The objective of this investigation was evaluate the association of the occupational risk and the sperm DNA damage assessed by the Comet assay, based on a descriptive model.

**Methods:** A total of 137 patients participated in the study, who were surveyed. The patients were grouped into three categories: Low-risk (office staff, traders, inspectors, stevedores and teachers), Medium-risk (athletes, masons, carpenters, machinery operators, chemical merchants, transport personnel and other activities; motorized police; and others) and High-risk (cooks, taxi drivers, farmers, welders, electricians and chemical workers). Individual cell agarose gel electrophoresis or Comet assay was performed.

**Results:** It was observed that patients with High-risk occupations have greater sperm DNA damage than patients with Low-risk occupations, specifically the percentage of tail intensity was  $44.72 \pm 16.07$  and  $15.55 \pm 16.51$  respectively ( $p=0.0001$ ). In addition, Low-risk patients showed a percentage of tail intensity of  $12.04 \pm 15.33$  for

normozoospermic and  $20.48 \pm 17.12$  for patients with 1 or more altered seminal parameters ( $p=0.0175$ ).

**Conclusion:** High-risk occupations showed greater sperm DNA damage which may play a negative role in reproductive health. This is concordant with the low clinical pregnancy reported for such sperm by our group and others.

## P-25. Assessing embryo quality to predict pregnancy

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**Objective:** To describe and compare pregnancy rates (positive HCG) between graded embryos after fresh single embryo transfer. The goal of in vitro fertilization (IVF) is to maximize live birth rates with the use of single-embryo transfer. Morphologic evaluation of embryos has historically been the method of choice to select the best embryo for transfer, however, this method has limitations.

**Methods:** Design: Retrospective study performed from 2017-2018 in two private clinics using the IVF laboratory located at Centro de Esterilidad Montevideo (CEM), Uruguay. Patients: Patients who underwent fresh single blastocyst transfers were included. Blastocyst morphologic grading was performed on day 5 or day 6 of embryo development. Embryos designated into the following groups: good (3-6 AA, 3-6 AB, AND 3-6 BA), average AND poor (2-6BB and 2-6 BC and 2-6 CB, early blastocyst).

**Results:** A total of 738 cycles were included. 81 cycles ended in fresh single embryo transfer. 30 patients had a positive HCG (37%), 51 patients (63%) had a negative result. From the group of positive HCG, 22 embryos (81%) were good, from the non-pregnant 26 embryos (51%) were good. Our data shows that pregnancy rates are significantly lower ( $p=0.0193$ ) after transferring average and poor quality embryos. When comparing demographic data, the difference between: age, BMI, AMH, number of mature oocytes and number of fertilized oocytes between groups (positive and negative beta) had no significant difference.

**Conclusion:** Morphologic evaluation of embryos is still critical specially to determine average and poor quality embryos which are less likely to achieve pregnancy.

## P-26. hCG as a predictor of pregnancy evolution in patients undergoing IVF

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**Objective:** Human chorionic gonadotropin (hCG) levels in early pregnancy are predictive for pregnancy outcome after an embryo transfer (ET), however prognostic values are not consistent due to the different timing of the hCG sampling. The aim of this study is to establish a cutoff value of hCG as a predictor of a viable pregnancy, 10 days after performing a blastocyst ET.

**Methods:** A cross-sectional, retrospective study was carried out in *in vitro* fertilization (IVF) cycles performed in the "Centro de Esterilidad Montevideo" between 2017 and 2018. Cycles included were fresh ET and frozen-thawed ET of blastocyst stage embryos that ended with a positive pregnancy test. Pregnancy was defined as a rise in hCG above 20 IU/L. All measurement of hCG levels were performed 10 days after the ET and hCG values were performed at the same center with an IMMULITE 2000 analyzer using the corresponding assay. A transvaginal ultrasound was performed on week 6 to confirm pregnancy. Pregnancies through preimplantation genetic test (PGT) were excluded. A probable viable pregnancy was defined as a pregnancy greater than 12 weeks of gestational age, and a non-viable pregnancy as a positive hCG blood test with the consequent results: early miscarriage, biochemical pregnancy and ectopic pregnancy. Biochemical pregnancy was diagnosed if no sign of pregnancy was evident on ultrasound examination. Miscarriage was defined as pregnancy loss after ultrasonic visualization of a gestational sac. Ectopic pregnancies were diagnosed by transvaginal ultrasound. Results were not adjusted by multiple pregnancy. The data obtained was analyzed with the use of software jupyter-notebooks, with python 3.6 as the main tool for the development of descriptive statistics. A level of statistical significance  $<5\%$  ( $p$ -value  $<0.05$ ) was used.

**Results:** A total of 241 cases were included. Patients were classified according to their evolution in:- Viable pregnancy/Good evolution: Pregnancy greater than 12 weeks of gestational age and pregnancy resulting in live birth.- Non-viable pregnancy/Bad evolution: Patients with the following results: Miscarriage, biochemical pregnancy and ectopic pregnancy. Out of the 241 cases, 161 patients presented viable pregnancies versus 80 patients with non-viable pregnancies; 43 miscarriages, 32 biochemical pregnancies, 5 ectopic pregnancies. An optimal hCG value was proposed as a predictor of pregnancy prognosis, comparing initial hCG values with the final outcome of the case. Cutoff values were proposed every 20 mIU/mL from values of 80 mIU/mL up to 360 mIU/mL. For each value the Recall and the Precision were defined and graphed in a "PR" curve:- Recall; percentage of total non-viable pregnancies that have a value lower than the reference value.- Precision: percentage of true positives obtained with the established reference value. The optimal cutoff value calculated, where the proportion of true positives is very high with respect to the amount of false positives, corresponds to 140 mIU/mL, statistically significant;  $p < 0.000000$ . There was no statistical difference in hCG levels between fresh and frozen cycles.

**Conclusion:** Considering that there is a statistically significant relationship between hCG values  $<140$  mIU/mL and non-viable pregnancies, an hCG value greater than or equal to 140 mIU/mL 10 days after performing a blastocyst stage ET can be used as a predictor of a pregnancy with a good prognosis during the first trimester, helping to better advice and monitor patients.

## P-27. Characterization of the Uruguayan semen donor population

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**Objective:** To evaluate the semen donor population of Uruguay through comparing data of successive samples banked by the same donor and the analysis of their biometric and anthropometric characteristics. Several studies have reported a global decline in seminal quality over the years, however results are contradictory, and some authors do not observe this trend. Sperm donation was officially established in Uruguay in 1989 since Fertilab opened a heterologous sperm donor program.

**Methods:** A total of 3449 ejaculated samples collected from 71 donors, cryobanked between 1989 and March 2017 at Fertilab were analyzed.

**Results:** The mean age of 23.90±3.98 years, average weight of 74.95±1.09 kg and mean height of 1.78±0.06 m. Most of the donors trace their origin to Europe (74.65%, 53/71) and 66.19% have a level of education higher than secondary school. Regarding semen parameters, on average each donor banked 48.57±3.99 samples. We observed differences along time of two parameters sperm concentration and semen volume. The average of sperm concentration was 85.70±0.70 x 10<sup>6</sup>/ml and semen volume were 3.03±0.02ml. Sperm concentration declined, while semen volume increased significantly over the 28-year period.

**Conclusion:** The present study is in accordance with previous reports that observe a decline in sperm count over the last years. However, no differences were observed on total sperm per ejaculate due to semen volume values, reflecting no changes in sperm production over the years.

## P-28. Oxidative stress and their relationship with semen parameters and reproductive outcome of in vitro fertilization (IVF) cycles with donor eggs: A retrospective study

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**Objective:** To relate seminal parameters with the level of ROS and DNA damage. To assess the impact of oxidative stress and DNA damage on reproductive outcome of IVF cycles with donated oocytes. Mammalian sperm are characterized by their ability to generate reactive oxygen species (ROS) that include superoxide anion and hydrogen peroxide. The toxic effect of these free radicals on sperm has been described in numerous publications. The production of ROS can damage sperm DNA. The imbalance between antioxidant defense mechanisms and generation of ROS produces oxidative stress, which can alter numerous processes including spermatogenesis, fertilization and embryo development.

**Methods:** A retrospective descriptive study was conducted with data collected from 156 egg donated-IVF cycles carried out between 2016 and 2017 at CEM. Historical records of patients, who underwent semen analysis at Fertilab's Andrology Laboratory were reviewed. Measures of semen parameters that were closest to the IVF cycle date were

the ones considered for the study. The TUNEL technique (Terminal dUTP Nick End Labeling) was used to determine sperm DNA damage. ROS were determined by reducing the nitroblue reagent from tetrazolium to formazan and then staining with Wright's dye. Statistics: a non-parametric correlation test was performed among the variables studied. The Spearman correlation coefficients were determined in order to establish an association between the variables studied. A value of  $p < 0.05$  was considered significant.

**Results:** Regarding traditional semen parameters we found a significant positive correlation between percentage of normal forms and concentration, total sperm count, total motile sperm and fertilization rate (Total Group;  $n = 156$ ;  $p < 0.0001$ ). For the group of patients with functional semen analysis (Functional Group;  $n = 37$ ) a significant negative correlation was found between percentage of normal forms and levels of ROS ( $p = 0.016$ ) and between percentage of normal forms and sperm with fragmented DNA ( $p = 0.012$ ). Fertilization rate was negatively affected (Functional Group) by DNA fragmentation ( $r = -0.354$ ,  $p = 0.041$ ). Total sperm count and total motile sperm correlated positively with ongoing pregnancy rate (Total Group;  $p = 0.006$ ;  $0.016$ ).

**Conclusion:** Oxidative stress relates to semen quality; worse semen parameters correlate with greater oxidative damage. The preliminary results indicate that sperm DNA fragmentation and percentage of normal forms impact fertilization rate but might not affect pregnancy rates.

## P-29. A retrospective study of egg donation program: comparison outcome of fresh and vitrified donor oocytes

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**Objective:** The aim of this study was to compare laboratory and reproductive outcomes of the egg donation IVF programs (fresh vs vitrified oocytes). Two different programs of oocyte donation are run in our center. One where the cycle is carried out using fresh donated oocytes and another in which vitrified donated oocytes are assigned to egg donor recipients.

**Methods:** A retrospective descriptive study was conducted with data collected from 328 fresh oocyte donation cycles and 79 vitrified oocyte donation cycles carried out between 2016 and 2018 in Centro de Esterilidad Montevideo, Uruguay. Comparison between fertilization, blastocyst, and ongoing pregnancy (first transfer and cumulative) rates was carried out to analyze programs. Statistical analysis was performed using XLSTAT (Addinsoft). A non-parametric Kruskal-Wallis test was conducted for comparison of variables that did not follow normal distribution. To compare ongoing and cumulative pregnancy rate a Z-test was performed. Values of  $P$  less than 0.05 were considered statistically significant.

**Results:** Table 1. Significant differences in the number of inseminated oocytes (9.5 vs. 7.1) fertilization (74.6% vs. 70.0%) and blastocyst (63.9% vs. 52.2%) rates were found. Therefore, fewer transferable embryos are obtained in cycles where vitrified oocytes were used (2.5 vs. 4.6). No differences were found in pregnancy rates when first

transfer was considered (44.6% vs. 38.4%), suggesting that the embryos that reach blastocyst stage and are chosen as first transfer (either fresh or after a FET cycle) have the same potential regardless of the origin of the oocyte. When comparing cumulative pregnancy rates (75.95% vs. 49.18%), a significant difference was observed in the fresh oocyte program, largely due to the fact that the patients have more blastocysts generated, hence more chances to do a second or third transfer if needed.

**Conclusion:** Recipients of the donation program with fresh oocytes start with higher number of inseminated oocytes, which, in combination with higher fertilization and blastocyst rates, yields a larger number of embryos suitable to transfer. Patients undertaking this program are given the chance of having more than one transfer to achieve pregnancy. The program using vitrified oocytes permits more flexibility in coordination of donor/recipient patients and allows for a better and homogenous distribution of eggs, but should increase the number of assigned oocytes in order to achieve similar cumulative pregnancy rates to those of the fresh oocyte program. The egg donor vitrification program could be reserved for patients that do not wish to cryopreserve embryos.

Table 1. Outcome					
Outcome	Fresh		Vitrified		p value
Laboratory Parameters	Mean	SD	Mean	SD	
Number of oocytes received (n)	11.01	3.38	7.54	1.81	<0.0001
Number of inseminated oocytes (n)	9.48	3.23	7.14	1.72	<0.0001
Fertilization rate (%)	74.64	19.33	70.03	19.83	0.03
Mean number of transferable embryos	4.59	2.48	2.56	1.53	<0.0001
Blastocyst rate (%)	63.89	24.03	52.21	27.23	0,00
Reproductive Outcome	+ (n)/cycles (n)	%	+ (n)/cycles (n)	%	p value
Ongoing pregnancy rate*(First Transfer) (%)	133/298	44.63%	28/73	38.36%	0.329
Cumulative on-going pregnancy rate*(%)	180/237	75.95%	30/61	49.18%	<0.0001

\* Ongoing pregnancy rate considers deliveries and positive ultrasounds to date.

## P-30. Outcome after late rescue Intracytoplasmic Sperm Injection

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**Objective:** The aim of this study is to report reproductive outcomes of ICSI rescued cycles. The average fertilization

rate after conventional in vitro fertilization (IVF) procedures is around 60-70%. However total fertilization failure (TFF) in patients with normal semen parameters may still occur. The proportion of TFF has been reported to be from 3.5% to as high as 15%-20% (Beck-Fruchter *et al.*, 2014). Total fertilization failure leaves the medical team with limited alternatives. The first is to cancel the current treatment cycle and to offer intracytoplasmic sperm injection (ICSI) in the subsequent cycle. The second alternative is to provide rescue ICSI in the current cycle (Nagy *et al.*, 1993). However, attempts to rescue unfertilized oocytes by ICSI when they are around 1 day old have yielded poor results. Poor quality embryos, higher 3PN rates, higher incidence of chromosomal abnormalities and low pregnancy rate have been reported (DeUgarte *et al.*, 2006).

**Methods:** A retrospective analysis was conducted with data collected from IVF cycles with TFF which were rescued by ICSI from January 2016 to December 2018 in our laboratory. Metaphase II 1-day-old unfertilized oocytes were collected from 27 patients. For the rescue ICSI, no additional treatment was applied to the oocytes or to the semen samples. A single spermatozoon was injected into the cytoplasm of each of these oocytes 20-30 h after ovum retrieval. Injected oocytes were observed at 16-18 h and 42-44 h after rescue ICSI was performed. Normal and abnormal fertilization rate, mean number of embryos transferred, clinical pregnancy and delivery rate were assessed.

**Results:** A total of 27 rescue ICSI cycles were performed injecting 206 metaphase II 1-day-old unfertilized oocytes. Normal fertilization rate was 52% (105/206) and abnormal fertilization rate (1PN+3PN) was 10.6% (22/206). Considering the normally fertilized oocytes, 27% (28/105) derived in transferable embryos. A mean of 1.67 embryos were transferred to 12 patients who underwent either fresh (n=2) or frozen/thawed embryo transfers (n=10). Regarding reproductive outcomes, pregnancy rate was 83% (10/12). To date, there are 4 ongoing pregnancies, 1 miscarriage and 5 deliveries of 6 healthy babies - one pair of twins and four singletons.

**Conclusions:** From this study it can be concluded that 1-day-old metaphase II oocytes which have failed fertilization after conventional IVF procedures could be fertilized by rescue ICSI with an acceptable pregnancy and delivery rate. This technique should be considered an effective alternative for couples having TFF, which otherwise would have lost the cycle.

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