# 2017 AAB Conference and CRB Symposium

# Abstracts



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#### **Analysis of Multiple PGS Variables**

#### Chapman C., Magno E., Oravec I., Pantos M., Varghese S., Wagner, Y. Reproductive Medicine Institute Oak Brook, Illinois

**Objective:** To investigate the influence of multiple variables such as culture medium, testing center and biopsy technician on euploidy rates in our program.

Design: Retrospective, randomized, sibling study in a private assisted reproductive technology program.

**Materials and methods:** A retrospective review of fresh PGS cases performed in the year 2016 was conducted. Patients with single gene disorders and/or translocation cycles were excluded. This program routinely splits sibling oocytes into two different culture media post-ICSI. All embryos underwent laser assisted zona hatching on day 4 utilizing a 100µs pulse to drill a small narrow opening through the zona pellucida. Biopsies took place on day 5 or day 6 of development when the blastocyst had several or more cells protruding out of the zona pellucida. Biopsies were conducted utilizing a multi-pulse laser. Biopsied samples were immediately fixed/loaded as per the protocol of the third-party testing center. Samples were sent to the third-party testing centers via priority FedEx.

**Results**: A total of 137 patients had 791 embryos biopsied (N=395 from medium A and N=396 from medium B) for euploidy evaluation by four different technicians. A targeted platform was utilized in 77 patients and a randomized platform was utilized in 60 patients.

	А	В	С	D	Р
Media	38.4%	44.4%			NS
	(152/395)	(176/396)			
Testing Center	47.1%	33.7%			NS*
_	(219/465)	(109/323)			
Technician	36.1%	13.3% (4/30)	51.1%	49.34 %	NS*
	(128/355)		(109/213)	(75/152)	

**Conclusions**: It has been suggested that euploidy rates can be influenced by culture conditions. In our program, we routinely split sibling oocytes post-ICSI into two different culture media. Looking at the euploidy rate independent of usual variables such as age patient diagnosis showed no difference in the percentage of embryos that were euploid in either Medium A or Medium B. Targeted or randomized sequencing is often debated. Our program routinely uses third party vendors that utilize either targeted or random sequencing. Our data show no difference in the amount of euploidy diagnosed when comparing platforms. It has been suggested that experience of the technician can influence the diagnosis of euploidy. We showed that technician can indeed be an influence but this did not reach significance.

Disclosures: KOL speaker Irvine Scientific

Funding: None

#### \*PENDING COMPLETED STATISCTICS

#### Comparative Human Blastocyst Repeat Vitrification (rVTF): Effect of Device Type (Cryolock versus microSecure) by Solution Used (DMSO/EG versus Glycerol/EG) on Postwarming Viability

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**Objective:** The VTF of human embryos has evolved into a highly reliable and efficient process in most ART laboratories worldwide, achieving over 99% survival and >70% implantation rates following blastocyst biopsy, cryopreservation and single euploid embryo transfer in our laboratory (see 2014-15 CDC Stats). Yet, substantial inter-lab variation still exists, primarily mediated by the introduction of more than 15 open and closed device systems into the IVF industry. The purpose of this investigation was to better understand the interrelationship between cryophysical and chemical factors on the efficacy of blastocyst VTF.

**Design:** Research consented, an euploid blastocysts (n=80) were randomly assigned to a 2x2 factorial design: open-Cryolock (CL) or closed-microSecure ( $\mu$ S) VTF devices, by EG/DMSO (15%/15%) or Glycerol/EG (G/EG; $\geq$ 7.9M) cryoprotective solutions. Differences in survival percentage and 24h development were statistically compared by Chi-Square analysis (p<0.05).

**Materials and Methods:** Vitrified blastocysts (AA to BB quality) were subjected to standard, rapid  $\mu$ S-VTF warming and then cultured in Global medium under tri-gas, humidified conditions (37°C). Upon equilibration (<2h), blastocysts were randomly assigned to a rVTF treatment group. All blastocysts were then vitrified using a standard device-solution VTF protocol, and subsequently warmed, re-equilibrated and cultured for 24h. Assessment of embryo survival was performed at 0h, 2h, and 24h (i.e., continued development) post-warming for all treatment groups.

**Results:** When comparing device-solution treatments, no significance was observed among blastocyst survival and 24h development (see Table). Furthermore, no differences in combined development occurred by cryoprotective agent (CPA: G/EG-80% vs EG/DMSO-85%), but tended to be 10% higher using the  $\mu$ S –VTF system (87.5%).

VTF device x solution:	CLxEG/DMSO	CLxG/EG	µSxEG/DMSO	µSxG/EG	P-value
# Warmed	20	20	20	20	
Oh Survival (%)	20(100%)	20(100%)	20(100%)	20(100%)	N/S
2h Survival (%)	19(95%)	18(90%)	19(95%)	19(95%)	N/S
24h Development (%)	17(85%)	14 (70%)	17(85%)	18(90%)	N/S

**Conclusions:** The type of CPA by device revealed no significant outcome differences. Less stable EG/DMSO solutions were equally effective independent of cooling/warming rate potential. In this study, human blastocysts have proven again to be highly resilient to rVTF, even when faced with CPA cross exposure. From a user perspective,  $\mu$ S -VTF clearly provided greater LN<sub>2</sub> and device handling safety. Additional trials and increased sample sizes are needed to fully discern the treatment effects of differential CPA exposures and device systems.

**Disclosures:** MC Schiewe developed µS-VTF without any commercial interests.

Funding: Support in-full by internal Ovation Fertility funds.

#### Elective Single Embryo Transfer (eSET) and Embryo Morphometric Phi in Term Pregnancies

Roudebush, W.E.,<sup>1</sup> Williams, S.E.,<sup>1</sup> Deaton, J.L.,<sup>2</sup> Wininger, J.D.<sup>2</sup>

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**Objective:** In order to optimize ART implantation success, embryologists typically select a preimplantation embryo(s) based on the embryos grade at the time of transfer. This embryonic grading is routinely a combination of objective and subjective assessment methods. However, this objective/subjective combination has been only minimally linked with successful implantations. Recently, significantly higher implantation rates have been observed utilizing novel embryo morphometric (embryometric) analyses that examine blastocyst length-to-width ratio or ICM-to-total surface area ratio that approximates phi ( $\theta$ =1.618, aka the Golden Ratio). To further explore the potential of embryometric phi analysis in ART, in particular in an elective single embryo transfer (eSET) program, we further evaluated these ratios in positive term pregnancy outcomes.

Design: Retrospective study in an academic assisted reproductive technology program.

**Materials and Methods:** Length-to-width (Word Draw) and ICM-to-total area (tpsDIG2; life.bio.sunysb.edu/morph/) were determined in day 5 blastocysts and phi ratios calculated for phi of day-5 blastocysts in term eSET deliveries. Calculated phi ( $\theta$ ) ratios [ $\theta_1 = (L^*W)/L$ ;  $\theta_2 = (TA*ICM/TA)$ ; and  $\theta_3 = (\theta_1+\theta_2)/\theta_1$ ] were compared by analysis of variance (ANOVA) and all pairwise multiple comparisons by the Tukey test (SigmaPlot for Windows v13.0.0.83).

**Results:** A total of 30 term pregnancies resulting from day-5 blastocyst eSET's were evaluated. In the length-to-width group,  $\theta_1$  ratios ranged from a low of 1.560 to a high of 2.109, with a mean of 1.870 ( $\pm$  0.026 SEM). In the ICM-total area group,  $\theta_2$  ratios ranged from a low of 1.072 to a high of 1.311, with a mean of 1.116 ( $\pm$  0.009 SEM). In a third phi ratio ( $\theta_3=\theta_1+\theta_2/\theta_1$ ) group,  $\theta_3$  ratios ranged from a low of 1.530 to a high of 1.688, with a mean of 1.600 ( $\pm$  0.009 SEM). ANOVA revealed a significant (P<0.001) difference in the means between the 3 groups. All pairwise comparisons (Tukey test) were also statistically (P<0.001) significant.

**Conclusions:** Embryometric analysis of blastocyst stage embryos have been previously found to provide a more objective assessment than traditional objective/subjective morphological approaches and are a significant aid in blastocyst selection for eSET. Of particular note,  $\theta_1$ ,  $\theta_2$  ratios and  $\theta_3$  ratios were found to be 115.6%, 69.0% and 98.9% of the Golden Ratio, respectively. Thus demonstrating that when the two morphometric ratios were combined into a third generation embryometric ratio, the resulting  $\theta$  ratio provided a greater aid in identifying the "ideal" blastocyst for eSET

Disclosures: None

Support: None

#### Embryo Culture Conditions are Superior During Uninterrupted Incubation – Randomized Controlled Trial

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**Objective:** To compare the culture characteristics of human pre-implantation stage embryos during uninterrupted culture in a time lapse (TL) incubator versus the conventional model of interrupted culture in standard incubator (SI) under low oxygen tension using a single step media.

Design: A parallel group prospective randomized controlled trial

**Materials and methods :** 221 patients under 35 years, 124 patients between 36-39 and 86 patients above 40 years were randomized and cultured either in a standard incubator or in a time lapse incubator (uninterrupted incubation). The patients were distributed into 1:1 ratio in the three age groups. The quality of days 2, 3 and 5 embryos were recorded. The pregnancy was considered positive if foetal heart beat was detected ultra-sonically weeks 7 after embryo transfer.

Data was analyzed using Student's *t*-test or Chi-square for trends. Two-tailed *p*-value < 0.05 was considered significant, all data analysis were performed using Microsoft Excel 2010.

**Results:** The fertilization rate, development of days 2, 3 and 5 embryos, and the clinical pregnancy rates were similar in both groups but there were differences between age categories within the two groups demonstrating less efficacious embryonic development with progression of age which appeared more pronounced in conventional incubator indicating its inferiority over uninterrupted incubation. The primary end point was percent of good quality blastocyst per randomized patient.

The proportion of fertilized oocytes (normal fertilizations) and of embryos that developed on days 2, 3 and 5, and the clinical pregnancy rates were mostly statistically similar in the three age groups in both TL and the SI incubators although the values were lower for embryos generated in the SI incubator. However when all age groups were combined significant differences between the uninterrupted and interrupted culture conditions immerged on days 3 (p=0.0112) and 5 (p=0.0027) of culture with all other parameters being similar which suggested the uninterrupted culture to be superior to the interrupted culture for the generation of better quality embryos.

**Conclusion:** Culture conditions for human embryos during uninterrupted incubation under low oxygen tension appears superior to conventional incubation.

**Disclosure:** Nothing to disclose.

Funding: None.

#### Expanding Human Blastocyst Expansion Takes Longer to Expand Compared to Expanded Blastocysts After Warming On Biopsied and Non-Biopsied Embryos: A Time Lapse Analysis.

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**Objective:** Blastocoel expansion has been shown to be an important indicator of human embryo viability and implantation potential which may be affected by mechanical manipulations like assisted hatching and embryo biopsy. Current clinical practice uses trophectoderm biopsy to screen for genetic abnormalities, but may introduce additional mechanical stressors to the preimplantation embryo. The present study seeks to understand if embryo stage and biopsy modifies the rate of blastocoel expansion after warming.

Design: Observational study performed in an academic medical center.

**Materials and Methods:** Day 5 embryos were vitrified and warmed at same center for frozen embryo transfer. The effect of biopsy on the rate of expansion was compared to controls (no biopsy). Warmed embryos were placed in Esco Miri<sup>®</sup> Time Lapse incubator and photographed every 5 minutes. Recording time was calculated starting from the first picture and ended after the blastocoel cavity was fully expanded. Embryo diameter from the first and last picture were measured at three times points using ImageJ and averaged. Rate of expansion was determined by the difference in length over time and statistical significance was calculated using Student's T-Test.

**Results:** There is no statistical significant difference between the rate of re-expansion of biopsied  $(0.135\mu m/min)$  and non-biopsied embryos  $(0.168 \mu m/min)$  regardless of their initial expansion stage (p>0.05).

		Grade 3*	Grade 4/5*	
Groups	n	(expanding)	(expanded)	Significance
Expansion rate of		n: 9	n:28	
non-biopsied embryos	37	rate: 0.100 µm/min	rate: 0.190 µm/min	(p<0.05)
		SD: 0.096	SD: 0.132	-
Expansion rate of		n: 11	n: 16	
biopsied embryos	27	rate: 0.095 µm/min	rate: 0.162 µm/min	(p=0.06)
		SD: 0.057	SD: 0.121	_
Average expansion rate of		n: 20	n: 44	
embryos regardless	64	rate: 0.097 µm/min	rate: 0.180 µm/min	(p<0.01)
of biopsy		SD: 0.075	SD: 0.128	

Table 1.

\* Gardner's criteria.

**Conclusion**: Our data suggests that expanding blastocysts exhibit a longer rate of expansion compared to expanded blastocysts (Table 1). This data additionally suggests that biopsy does not alter the rate of re-expansion. However we observed that more expanded blastocysts re-expand at an almost significantly faster rate even after undergoing biopsy, but a larger group of embryos are necessary to re-evaluate this category. Future studies will focus on utilizing expansion rate as a predictor of implantation success to provide better clinical scheduling management of frozen embryo transfer for biopsied embryos.

Disclosures: Nothing to disclose | Funding: None

#### Impact of MTHFR Isoform C667T on Fertility Through Sperm DNA Fragmentation Index (DFI) and Sperm Nucleus Decondensation (SDI)

Clement, A.,<sup>1</sup> Cornet, D.,<sup>2</sup>, Cohen, M.,<sup>3</sup> Amar, E.,<sup>4</sup> Clement, P.,<sup>1</sup>, Menezo, Y.<sup>5</sup> <sup>1</sup>Laboratoire Clement, Ile de France, Paris, France. <sup>2</sup>Clinique de la Muette, Ile de France, Paris, France. <sup>3</sup>Natecia, Rhone-Alpes, Lyon, France. <sup>4</sup>American Hospital of Paris, Ile de France, Neuilly sur Seine, France. <sup>5</sup>London Fertility Associates, London, London, United Kingdom.

**Objective**: To study if MTHFR isoforms are involved in the subfertility pathologic aspects through sperm nucleus structure parameters (DFI and SDI).

**Design**: This is a retrospective study involving subfertile couples controlled for C677T isoforms in an IVF unit. Male partners were tested for DFI and SDI during the year 2015-2016. Eighteen patients were controlled as carrying the isoform on a heterozygote state. Nine were found to be homozygote for the C677T isoform: these patients faced 3 to 9 IVF/ICSI failures and/or miscarriages. Their DFI and SDI were compared to our control group involving more than 1400 patients.

**Materials and methods**: MTHFR isoforms detection is now part of our assessment in couples after repeated failures of IVF/ICSI failures. Determination of C677T isoform was performed with Real Time Polymerase Chain Reaction. DFI and SDF is measured using Acridine orange/Flow cytometry according to a technique recently described (Hamidi).

**Results**: In our general sub-fertile population, 40% of the patients have a DFI >20% and 23% have a SDI>20%; 20% being considered as the threshold value. Out of the 18 heterozygote patients, 3 have high DFI and SDF (16%). For the homozygote patients, 8/9 have pathological values: two have both high DFI and SDI (average: 41% and 31% respectively). 4 have an isolated high SDI (average: 29%), 2 have only a high DFI only (average: 28%). One patient has correct DFI and SDI values.

**Conclusions**: The homozygote MTHFR C677T induces pathologies of nucleus structure measured by SDF and DFI. Therefore MTHFR polymorphism could increase the male infertility risks. In case of repeated ART failures, patients with very high DFI/SDF could be tested for MTHFR and treated by 5 Methyl tetrahydrofolic acid, a compound of the 1-CC downstream the MTHFR.

Disclosure: Nothing to disclose.

Funding: None.

#### Distribution of DNA Fragmentation Index (DFI) and Nuclear Decondensation Index (SDI) in a Population of More Than 1400 Subfertile Couples

Clement, P.,<sup>1</sup> Amar, E.,<sup>2</sup> Cornet, D.,<sup>3</sup> Jacquesson, L.,<sup>4</sup> Clement, A.,<sup>1</sup> Menezo, Y.<sup>5</sup>
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**Objective:** To study the distribution of DFI and SDI values among a population of subfertile couples.

**Design:** We conducted a prospective controlled trial. Every patients having a DNA structure analysis between January and December 2016 were included in our study. The link between classical sperm parameters, DFI and SDI were also analysed.

**Materials and Methods:** The male patients were included based on recurrent ART failures and a real difficulty to conceive. DFI was measured via terminal deoxynucleotidyl transferased UTP nick end labeling (TUNEL) assay. SDI was measured using aniline blue.

**Results:** 1417 couples were included. We determined 3 zones: green zone: no male factor, grey zone: male factor cannot be excluded, red zone: strong male factor. Using DFI: green zone if DFI<20% (concerned 60.0% of the population: 848/1417 male); grey zone if DFI<25% (9.0%: 129/1417); red zone if DFI>25% (31%: 439/1417). Using SDI: green zone if SDI<20% (77%: 1094/1417); grey zone if SDI<25% (10%: 148/1417); red zone if SDI>25% (12%: 175/1417).

**Conclusions:** Based on the threshold values, 40% of the patients tested had a DFI over 20% and 22% had a SDI over 20%. Being able to identify patients with nucleus condensation anomalies will allow us to treat those patients efficiently in order to reach lower DFI and SDI values and therefore improve the ART outcomes.

Disclosure: Nothing to disclose.

Funding: None.

#### Effects of Supplementation of Antifreeze Proteins on Follicular Integrity of Vitrified-Warmed Mouse Ovary: Comparison of Two Types of Antifreeze Proteins and Their Combination

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**Objective:** To analyze the effect of supplementation of two types of antifreeze proteins (AFPs) and their combination into vitrification-warming solution on follicular integrity of vitrified-warmed mouse ovaries.

**Method:** Ovaries (n = 154) were obtained from seventy seven 5-week-old BDF1 female mice and were vitrified by using ethylene glycol and dimethyl sulfoxide with supplementation of 10 mg/mL Flavobacterium frigoris ice-binding protein (FfIBP), 10 mg/mL Type III AFP or their combination. Ovarian sections were examined by light microscopy after H&E staining and follicular intactness was assessed as a whole and type of follicle. Apoptosis within whole follicles was detected by TUNEL staining.

**Results:** The proportion of overall intact follicle was significantly higher in Type III AFP-supplemented group (60.5%) and combination group (62.9%), when compared with non-supplemented control (43.8%; P < 0.05 for each). The proportion of intact primordial follicle was significantly higher in FfIBP-supplemented (90.0%), Type III AFP-supplemented (92.3%) and combination group (89.7%), when compared with non-supplemented control (46.2%; P < 0.05 for each). The proportions of non-apoptotic follicle were all similar between four groups.

**Conclusion:** Supplementation of FfIBP, Type III AFP or their combination into vitrification and warming solution is equally beneficial on the preservation of primordial follicles when mouse ovaries are vitrified.

Key words: ovary, vitrification, antifreeze protein, fertility preservation

#### Frozen Embryo Transfer Instead of Fresh Embryo Transfer Can Increase Pregnancy and Implantation Rates and Reduce Biochemical Pregnancy In Advanced Maternal Aged Women

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**Objective:** To compare outcomes of ongoing pregnancy rates, clinical pregnancy and biochemical pregnancy rates in women aged 35 years or older undergoing a fresh vs a frozen embryo transfer.

#### **Design:** Retrospective study

**Materials and Methods:** 279 embryo transfer cycles were analyzed with fresh embryo transfer (n=172) or frozen embryo transfer (n=107) in women aged 35 years or older. Egg donor/gestational carrier or PGS/PGD cases were excluded. (Clinical) Pregnancy per transfer, implantation rate, and biochemical pregnancy per pregnancy case in fresh and frozen embryo transfer cycle were compared. Data were described as the average  $\pm$  standard deviation or percentages. The statistical analysis was performed using Student's t test, chi-square, or z-test. A *p* value of <0.05 was considered statistically significant.

#### Results

#### Table1. Pregnancy rate by age group

	≥ 35 yrs		≥ 38	yrs
	Fresh (n=172)	FET (n=107)	Fresh (n=89)	FET (n=39)
Age	37.98±2.49	37.38±2.3	39.92±1.84	39.79±1.77
Number of embryos transferred	2.06±0.7	1.66±0.49*	2.19±0.83	$1.58 \pm 0.55*$
Pregnancy rate per transfer	52.9%	62.6%	42.7%	59.0%*
Clinical pregnancy rate per transfer	40.1%	57.9%*	33.7%	56.4%*
Implantation rate	24.9%	41.0%*	18.5%	35.5%*
Biochemical pregnancy rate	24.2%	7.5%*	21.1%	4.3%*

\*The result is significant at p < 0.05.

**Conclusions:** Frozen embryo transfer is a better option than fresh embryo transfer in women aged 35 years or older with improved pregnancy rates and lower percentage of biochemical pregnancies.

#### **Functional Time of Two Types of Cryotanks in Three Different Orientations**

Pomeroy, K. O. PhD, HCLD(ABB), and LoManto, B. MS The World Egg Bank - Phoenix, AZ

**Objective:** Determine the hold times for two types of dry liquid nitrogen tanks at three different orientations.

**Design:** Prospective 2 X 3 X 3 factorial

**Materials and Methods:** Two tanks types, a Cryoport and an MVE (SC4/3V) were placed in three different orientations, upright, upside down and on their side. They were placed in these positions after standard filling with liquid nitrogen as if they contained specimens (plugs and caps on) and were fitted with thermocouples to measure their internal temperature. The time until the tanks reached the temperature of - 110 C (the theoretical glass transition temperature) was determined. Three replicates of each orientation/tank type were performed.

**Results:** MVE tanks lasted longer than Cryoport tanks in all cases. When upright, MVE lasted over one week longer to the glass transition temperature than Cryoport tanks (20.8 days vs 12.2 days), despite the higher capacity of the Cryoport tanks (4.5 liters vs 3.5 liters for MVE). MVE lasted on average 4 days longer on the side or upside down compared to Cryoport tanks. Laying the tanks upside down reduced the holding time to about one third (34% for MVE vs 38% for Cryoport). It is interesting that tanks placed on their side did almost as poorly as upside down tanks. Upside down tanks had significantly shortened holding times to about 6 days for Cryoport tanks (51%) and 10 days for MVE tanks (49%).

When stored upright, the the Cryoport tank consumed over twice as much liquid nitrogen (334 ml/day) compared to the MVE (160 ml/day).

**Conclusions:** MVE tanks provide a longer holding time when compared to Cryoport tanks regardless of their orientation. MVE tanks are more efficient in liquid nitrogen consumption compared to Cryoport tanks, consuming almost half as much liquid nitrogen. Holding time is almost cut in half when either tank is placed on their sides.

Disclosures: none

**Funding:** The World Egg Bank

#### How Semen Parameters Affect Ploidy Status of Embryo: A Time-lapse Study

Dulaj D.,<sup>1</sup> White A.,<sup>1,2</sup> Dogan S.,<sup>1</sup> Fakih M.,<sup>1</sup> Shamma N., <sup>1</sup> Hammoud A.,<sup>1</sup> Ayers J.,<sup>1,2</sup> and Khan I.,<sup>1</sup> <sup>1</sup>IVF Michigan, 37000 Woodward Ave #350 Bloomfield Hills, Michigan, United States, 48304. <sup>2</sup>IVF Michigan, 3145 Clark Rd Ypsilanti, MI United States 48197

**Objective:** To investigate the effects of sperm concentration and motility on embryo morphokinetics and ploidy status.

Design: Retrospective cohort study.

**Materials and Methods:** Data were collected from randomly selected patients who used autologous fresh oocytes for their IVF-PGS cycles (n=56) during 2013-2016 in our clinic. We excluded those who used donor oocyte or testicular sperm extraction. Oocytes were incubated in Time-Lapse microscope (EmbryoScope: Vitrolife) following intra cytoplasmic sperm injection (ICSI). Following the biopsy either on Day 3 or Day 5, ploidy status of embryos was analyzed by Genesis genetics. These embryos were classified into two groups (n=243) based on their ploidy status (Group 1: Euploid embryos vs. Group 2: Aneuploid embryos) after excluding embryos with complex abnormality with no sex. Since ICSI was used for insemination, sperm morphology was also excluded from the data set. Data were analyzed using student t-test,  $\chi^2$ -test and Pearson correlation test.

**Results:** The mean differences of all parameters within groups were tabulated below. As expected, female age was different within euploid vs aneuploid embryos (p < 0.0001). We did not find any correlation between sperm concentration with PNa, t2, t3, cc2, t4, s2, tM (R= -0.0909, -0.0369, -0.0073, 0.0459, -0.0466, -0.0654, -0.0657, respectively). Likewise, there was no correlation between sperm motility and embryo morphokinetics (R= -0.0978, 0.1222, 0.1385, 0.0348, 0.099, -0.0581, -0.1086, respectively).

	Euploid Embryos	Aneuploid Embryos	p-values
n	118	125	
Female Age	34 ± 5.2	$37.5 \pm 4.3$	< 0.0001*
Sperm Concentration (10 <sup>6</sup> /ml)	$62.0 \pm 45.3$	$67.8 \pm 45.6$	0.324
Sperm Motility (%)	51.8±21.7	$52.8 \pm 20$	0.702
tPNA (time of PN appearance)	$9.2 \pm 2.7$	9.3±3.5	0.782
t2 (time of 2nd blastomere appearance)	26.7±5.3	27.7±7.8	0.230
t3 (time of 3rd blastomere appearance)	37.3±6.5	37.9±7.4	0.571
cc2 (time of 2nd cell cycle; t3-t2)	$10.7 \pm 4.2$	10.1±4.3	0.339
t4 (time of 4th blastomere appearance)	39.0±6.3	40.6±7.9	0.087
s2 (time of synchrony of 2nd cell cycle; t4-t3)	$1.7 \pm 3.7$	$2.8 \pm 4.9$	0.054
tm (time from insemination to formation of a morula)	90.5±10.9	90.2±11.5	0.832
Male (%)	52.5%	52.8%	0.5169
Female (%)	47.5%	47.2%	0.637
Ratio male/female	1.11	1.12	0.994

**Conclusions:** According to our findings, sperm concentration and sperm motility were similar within euploid vs aneuploid embryos. Therefore, we concluded that sperm concentration and sperm motility may not directly affect embryo morhokinetics and ploidy. We will improve this ongoing project by predetermining our groups based on semen parameters and by extending the dataset to confirm our findings.

Disclosures: Nothing to disclose

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#### Laboratory Evaluation of a Point of Care Molecular Test for the Detection of Influenza A and B

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**Introduction:** Rapid laboratory identification of respiratory viral agents is increasingly important due to the use of antiviral agents and the continued emergence of respiratory virus strains with the potentially for global impact. The Carolinas Medical Center uses PCR based testing for diagnostics on in-patient, transplant, and immunocompromised patients. In addition, culture and Direct Fluorescent Antibody (DFA) methods for the detection of respiratory viral agents are used for outpatients. Testing at the point of care (physician office laboratories [POC]) was discouraged due to the poor performance of rapid cartridge methods. Recently, PCR methods have become available that are touted as more sensitive than previous POC methods. In addition, these methods have a rapid time to result.

**Objective:** In this study we compared conventional PCR [(real time polymerase chain reaction assaysbead based DNA amplification tests (xTAG RVP FAST)] and a rapid PCR method designed to be used in the POC (Roche LIAT).

**Method:** All samples were banked previously confirmed strains or fresh collected using nasal wash, flocked swab NP, bronchial washes, or tracheal aspirate samples. The banked strains were confirmed as to their identity using in-house FDA cleared PCR methods or were obtained from the NC state public health laboratory. A total of 121 samples were tested. This included 94 strains of Flu A (seasonal flu strains and H1N1 strains) and Flu B. All negative Flu samples which were positive for other viral agents such as parainfluenzae, RSV, adenovirus, and metapneumovirus to assay the potentital of viral cross reactivity.

**Results:** Overall, the LIAT was tested on 94 positive and 25 Flu negative samples demonstrating a sensitivity/ specificity of 100% (95% CI 95-100%)/ 93% (95% CI=74-98.7%) respectively as compared to the xTAG RVP FAST assay.

**Conclusion:** The LIAT assay is an accurate and reliable method for detecting both FLU A and B infections and offers the advantage of detecting FLU at the point of care in a timely fashion.

#### Mathematics and Statistical Analysis for Embryo Selection Versus the Human Eye: Which One Bears More Predictive Value?

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**Objective:** The EmbryoScope<sup>TM</sup> has technology which captures embryo images which are annotated for key milestones in development. Mathematical and statistical analysis is used to assess these milestones and generate a score: Compare and Select (C/S) which is used to aid in embryo selection. The system does not have the capability of grading morphological parameters. Our program uses both C/S and morphology to select the best embryo for transfer.

**Design:** Retrospective study to determine if mathematical and statistical analysis is as predictive in embryo selection for transfer as morphological embryo grading.

**Materials and Methods:** A total of 180 embryos from 136 patients cultured in the EmbryoScope<sup>TM</sup> with known livebirth results are included in the study. Cell number on day 2 and 3, morphological grading and mathematical C/S score on day 3 and 5, as well as characteristics of inner cell mass (ICM) and trophectoderm (TE) on day 5 were analyzed.

**Results:** Among the embryos, 38 resulted in livebirth. Demographics and analytic parameters are shown in table 1. In addition, C/S score vs morphological grade on Day 3 (p=0.049) and day 5 ICM (p=0.006) by McNemar's test indicate increased sensitivity of C/S scores and that Day 5 ICM scores are more important than day 5 TE scores.

	+LB, competent no LB, incompetent				
Results	embryos	embryos	P value		
Ν	38	142			
Age	32.5 +/- 4.3	35.5 +/- 2.5	p<.0001		
# Embryo transferred	1.2 +/- 0.4	1.9 +/- 0.7	p<.0001		
# Cells on Day2	3.95 +/- 0.8	3.93 +/- 0.4	p=0.89		
Morphological grade on Day 2	0.6 +/- 0.9	0.6 +/- 0.6	p=0.68		
# Cells on Day 3	11.1 +/- 14.8	8.4 +/- 3.7	p=0.06		
Morphological grade on Day 3	0.38 +/- 0.38	0.64 +/- 0.53	p=0.0012		
Day 3 Compare and Select score	1.44 +/- 0.17	1.27 +/- 0.39	p=0.0002		
Morphological grade on day 5 ICM	1.03 +/- 0.16	1.15 +/- 0.43	p=0.011		
Morphological grade on day 5 TE	1.19 +/- 0.7	1.42 +/- 0.13	p=.18		
Day 5 Compare and Select score	2.03 +/- 0.2	1.92 +/- 0.26	p=0.020		

Table1. Comparison of competency of embryos using livebirth outcome

**Conclusions:** While morphological grading is still necessary in the process of embryo selection for transfer, mathematical C&S shows a higher sensitivity as a diagnostic tool for pregnancy outcome.

Disclosures: None

Funding: None

#### Normal Euploid Blastocyst Grown From a Thawed Day 2 Slow Frozen Embryo After 14 Years in Cryostorage

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**Objective**: Cryopreservation, extended embryo culture and preimplantation genetic screening (PGS) has given patients increased reproductive potential. Over the years, cryopreservation has changed from slow-freezing with ~40% survival to vitrification with significantly higher survival. Extended embryo culture to the blastocyst stage has become routine. PGS has changed from cleavage stage biopsies with FISH on limited chromosomes to trophectoderm biopsies with Next Generation Sequencing (NGS) of all 24 chromosomes. This case report describes a patient who after having one child in 2002 returned 14.3 years later and requested thawing, blastocyst biopsy and NGS evaluation of her previously cryopreserved Day 2 embryos.

**Design**: To report on the laboratory outcomes of a patient's request to evaluate her 14-year-old cryopreserved Day 2 cleavage stage embryos for genetic normality.

**Materials and Methods**: All cryopreserved Day 2 cleavage stage embryos were originally developed from oocytes aspirated in 2002. Fertilization was achieved by ICSI and 8 cleavage stage supernumerary embryos were cryopreserved via a 2-step Propandiol slow-freeze technique (Irvine Scientific, Irvine, CA). The embryos were thawed in 2016 using a slow thaw technique (Embryo Thaw – Irvine Scientific) and cultured for up to 5 days in single step media (CSCMC – Irvine Scientific). Trophectoderm biopsies were performed on hatching blastocysts of fair quality and with evaluation by NGS (Reprogenetics, Livingston, NJ).

**Results**: A 39-year-old female underwent an IVF cycle in 2002 with a transfer of a Day 2 embryo which resulted in a live birth of a healthy female. The 8 remaining embryos were frozen on Day 2 via slow-freezing. Fourteen years later she returned requesting PGS of her frozen embryos. Eight embryos were thawed, 7 survived and were cultured for up to 5 additional days. One out of 7 embryos developed into a hatching blastocyst of fair quality on Day 6 and 2 achieved this stage on Day 7, all 3 underwent trophectoderm biopsy. Four embryos were discarded due to poor quality. PGS results indicated 1 normal euploid male embryo, while the other 2 displayed aneuploidy.

**Conclusion**: A single euploid male embryo was diagnosed after thawing, extended culture and PGS testing of a 14.3 year stored slow-frozen Day 2 embryo. To our knowledge this is the longest cryopreserved embryo that has been successfully thawed, cultured to a blastocyst, had a trophectoderm biopsy and was screened as genetically normal by NGS. We await the patient's decision as to if she will proceed with a frozen embryo transfer.

Disclosures: None

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#### Sperm DNA Fragmentation – Does Age Really Matter?

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**ABSTRACT**: Male fertility declines with increase in age where every single delayed year accounts to a considerable difference. Impaired fertilization, poor embryo and blastocyst development, lower implantation rates and higher instances of miscarriage are the adverse effects of damages in sperm DNA, thereby affecting fertility. Factors contributing to sperm DNA damage can be both internal and external. Intrinsic factors include protamine deficiency, mutations that affect DNA compaction, and advanced paternal age. High levels of reactive oxygen species (ROS') are detected in the semen of infertile men which directly or indirectly influences DNA fragmentation. In addition, certain external factors such as heat, chemotherapy, radiation, and others are also associated with an increase in DNA damage. Cigarette smoking, genital tract inflammation, varicoceles, and hormonal deficiencies have all been found to be the factors of DNA damage. To top it all, age is a constant factor influencing DNA fragmentation and chromosomal abnormalities in the spermatozoa of infertile patients and fertile men. A research study conducted at ARC International Fertility & Research Centre in Tamil Nadu, India monitored Sperm DNA fragmentation in 192 infertile males and a comparison was made with the men's age, using Sperm Chromatin Structure Assay (SCSA) at ANDROLOGY CENTER in Tamilnadu for detecting broad range of DNA damage. The study found that DFI was better below the age of 40, as a result of which patients considering a delay in childbearing for social reasons or those seeking fertility treatments could be effectively counseled. Group A with 96 infertile men below age of 40 yrs, it was found that 44% of the men were affected by sperm DNA fragmentation and group B with 96 infertile men above age of 41 yrs, 63% of men were affected with a change in HDS that would result in negative pregnancy outcome. The variation proves the difference in age could make in sperm DNA fragmentation. Hence, age is found to be an important factor to be considered for male infertility.

#### Striving for One Embryo-One Baby: How the Integration of Vitrification and Preimplantation Genetic Screening (PGS) Technologies has Impacted Society

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**Objectives:** Optimization of embryo culture practices led to our highest success rates following fresh embryo transfer (ET) in 2010, with live birth rates (LBRs) per ET ranging from 75.3% for donor egg (DE) cycles to 53.2%. 55.1%, 36.8% and 20.7% for women <35yo, 35-37yo, 38-40yo and 41-42yo, respectively, transferring a mean of 2.0 to 2.6 embryos. These success rates were favorable, ranking in the upper 10<sup>th</sup> percentile nationally. However, multiple births were excessive at 28-70% when transferring 2 blastocysts. Through elective vitrification and adoption of blastocyst biopsy/PGS, we aimed to improve the quality of patient care by transferring fewer embryos, reducing miscarriages and increasing single healthy live births.

**Study design:** Retrospective analysis of the annual national report by the Centers for Disease Control (CDC) from 2010 to 2015. Our goal was to contrast industry trends regarding fresh DE-ET (control group) to autologous frozen ET cycles, in number of embryos transferred, implantation rate, and LBR/ET, and assess how our clinical practices improved the quality of patient care.

Materials and Methods: Our routine laboratory procedures involve: ICSI, group culture in Global<sup>™</sup> medium + 7.5% protein supplement, tri-gas incubation, transvaginal ultrasound-guided ET, and microSecure vitrification of blastocysts in non-DMSO I.C.E. solutions (Innovative CryoEnterprises, NJ) with or without blastocyst biopsy.

**Results:** Although the mean number of fresh DE embryos transferred decreased from 2.0 to 1.6 between 2010 to 2014, respectively, little change in LBR (55.8% to 56.8%) occurred in the USA. Conversely, the same mean cryopreserved embryo number yielded increased (P<0.01) implantation success (<34.3% to 43.7%), more singleton LBs (<31.9% to 36.5%) and higher total LBRs/ET (38.4% to 46.6%). With emphasis on PGS in 2014, our SCCRM affiliate clinic transferred 1.1 to 1.2 blastocysts/ET in women <35 yo, 35-37yo, 38-40yo and 41-42yo (n>250 cycles), achieving implantation rates of 77.9%, 61%, 60% and 84%, respectively, and 71%, 69.4%, 71.4% and 64.3% in 2015 (per CDC). Transferring 1.0 blastocyst/ET in women <35 yo in 2015, we produced the highest healthy singleton LBR (61.3%) reported by the CDC for any center.

**Conclusion:** The global adoption of highly reliable vitrification practices has facilitated significant, progressive improvements in IVF-FET outcomes. In conjunction with blastocyst biopsy/PGS, single euploid embryo transfer has optimized embryo utilization rates, and significantly elevated LBRs/ET in women under 38yo. Although the same trend was not significant on a per cycle basis in older women (38-42yo), it appreciably reduced the trauma/depression associated with pregnancy loss and reduced the time needed to achieve the desired outcome of healthy singleton births. Overall, vitrification and PGS allowed us to enhance ethical standards in patient care and improve the healthy singleton LBR.

**Disclosures:** JW and REA serve as educators for Illumina. MCS developed mS-VTF without any commercial interests.

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#### Wasting Resources in the Evaluation of Anemia: Overuse and Inappropriate Use of Laboratory Tests

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**Objective:** As the US Healthcare System transitions from a fee-for-service to a fee-for-value or qualitybased payment system, healthcare providers will be provided incentives to eliminate waste, overuse and inappropriate use of testing and treatment. Approximately 10% of total US health care spending is used on inappropriate (or overused) diagnostic testing and treatments. The Choosing Wisely Campaign has recruited most physician specialty organizations and many non-physician healthcare organizations to create their Top 5 List of overused tests and treatments. Anemia is a multifactorial condition associated with a variety of adverse outcomes. However, one of the authors of this paper (DLS) had noted the routine of physicians ordering the combination of iron studies (serum iron and/or ferritin and/or total iron binding capacity) and/or B12 and/or folate in the initial work-up of anemia. We arbitrarily drew our patient sample from all laboratory work collected between April 15, 2014 and January 15, 2015 (9 months).

**Design:** One group had a diagnosis of Anemia, unspecified (ICD-9 code of 285.9). The second group had some other Anemia diagnosis (ICD-9 codes of 280.0-285.8). The third group were patients with anemia diagnoses but no anemia. Among these subgroups, we studied laboratory testing patterns and results.

**Results:** Among the group of patients with some other type of anemia diagnosis (ICD-9 codes 280.0-285.8) some type of iron deficiency anemia accounted for 69% of cases (280.0-280.9). Twenty-six percent (26%) of cases were deficiency anemias (B12 and folate, 281.0-281.9), 1% were hereditary hemolytic anemias (282.0-282.9), 0.5% were acquired hemolytic anemias (283.0-283.9) and less than 0.5% were aplastic anemias (284.0-284.9). Among the 6,512 anemia patients, only 40% had abnormal serum iron, 17% had low ferritin levels, less than 1% had low B12, and 1.1% had low folates. Among another 6,416 patients with anemia diagnoses as defined above, 12% had abnormal iron, and less than 4% had low ferritin, less than 1% low B12 and 1.2% low folate.

**Conclusions:** These preliminary findings indicate that there is a clear opportunity to improve the ordering practices and use of the clinical laboratory in patients with anemia or suspected to have anemia.





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