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Abstracts

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ABSTRACTS – ORAL

ABSTRACTS – POSTER

Assessing Euploid Status in Day 5 or Day 6 Blastocysts Among Patients Whose Embryo Cohorts Undergo Both Normal and Delayed Blastulation

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Objective: Recent data demonstrate higher aneuploidy rates in day 7 embryos. It is unknown if increased aneuploidy rates are limited to specific embryos with slower development or if the presence of slower development indicates higher aneuploidy risk for a patient's entire embryo cohort. This study compares aneuploidy rates in women with some slow-developing embryos to those whose embryos blastulate only on days 5 and 6.

Design: Retrospective study

Materials and Methods: 21,237 embryos retrieved from 4,314 IVF cycles between January 2017 and March 2019 were evaluated. Embryos underwent blastocyst-stage preimplantation genetic testing via next-generation sequencing. Embryos were included from two groups. Group 1 was women whose IVF cycles yielded embryos with day 5 or 6 blastulation as well as delayed blastulation (day 7). Group 2 included women who only produced day 5 or 6 blastocysts.

Euploidy rates for day 5 or 6 blastocysts were compared between groups. Within Group 1, euploidy rate of day 5 or 6 blastocysts was compared to euploidy rate of day 7 blastocysts. A generalized mixed effects model determined statistical significance, with $p<0.05$ considered significant.

Results: Group 1 consisted of patients who had some embryos blastulate on day 7. The group had 3,416 embryos undergoing blastulation on day 5 or 6 and 1,012 embryos undergoing blastulation on day 7. Group 2 consisted of 16,809 embryos from women whose developing blastocysts expanded prior to the end of day 6. Overall euploidy rate for day 5 or 6 blastocysts from Group 1 was 56.0% (binomial 95% CI 54.3%-57.7%). Day 5 or 6 blastocysts from Group 2 had an overall euploidy rate of 58.1% (binomial 95% CI 57.3%-58.8%). There was no statistically significant difference in euploidy rates for day 5 or 6 blastocysts between groups ($p=0.79$).

Overall rate of euploidy for day 7 blastocysts from Group 1 was 40.6% (binomial 95% CI 37.6%-43.7%). Within Group 1, a significantly lower rate of euploidy was observed in day 7 blastocysts when compared to their day 5 or 6 blastocysts ($p<2.2e-10$).

Conclusions: Euploidy rates for day 5 or day 6 blastocysts were not impacted by the rate of development of other embryos within the same cohort. Each embryo's risk appears to be independent. These results indicate that patients who have some slow-developing embryos are not at increased risk of aneuploidy in their day 5 and day 6 embryos.

Disclosures: None

Funding: None

Assessing Mitochondrial Quantity and Ranking (Mitoscore) in Euploid Single Embryo Frozen Transfers Demonstrates a Lower Mitoscore is Significantly Better

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Objective: Mitochondria are cellular power houses acting as the principal site for ATP production. Mitochondrial function, mtDNA gene expression, and energy are required to undergo necessary cellular divisions in the formation of a high quality embryo. Unlike other cellular organelles, mitochondria contain their own DNA (mtDNA). It is known that mitochondrial functions are imperative during preimplantation development, however the quantity of mtDNA an embryo requires is unknown. Our current best practices for embryo selection include day of development, morphology grade and preimplantation genetic testing for aneuploidy (PGT-A). In this study we compared clinical pregnancy rates of our current protocols for embryo selection and any relationship to the Mitoscore ranking.

Design: Retrospective analysis of clinical outcomes as they associate with laboratory characteristics for embryo development, embryo quality and Mitoscore ranking.

Materials & Methods: During days 5-7 of embryo development, trophectoderm biopsy was performed on blastocysts by an embryologist using a laser to remove 3-5 trophectoderm cells for PGT-A. The biopsied cells are washed through buffer media, loaded into PCR tubes and frozen in a -20°C freezer until analysis. Whole genome amplification and next generation sequencing (NGS) using the Ion ReproSeq PGS Kit for 24 chromosome aneuploidy screening using the Ion Reporter software was performed. The genetic analysis uses complex algorithms to delineate mtDNA scores (Mitoscore). Frozen single euploid embryo transfer decisions were based on embryo developmental day and overall embryo quality grading criteria but did not include Mitoscore ranking. Pregnancy outcomes were analyzed and assessed as they associated with the Mitoscore number and its rank in the cohort.

Results: From this study we learned that using our current embryo selection criteria, excluding Mitoscores, resulted in only a 64.4% overall pregnancy rate. However, in embryos with Mitoscore values <25 we had a significantly improved pregnancy rate at 85.2% ($p<0.001$) and achieved the highest implantation at 90.9% with Mitoscores <20 (trending toward significance with a $p=0.067$) (Table 1).

Table 1: Mitoscore Values and Pregnancy Rates

Mitoscore Value	Pregnancy Rate
<20	**90.9% (10/11)[#]**
>20	**55.9% (19/34)[#]**
<25	**85.2% (23/27)[*]**
>25	**33.3% (6/18)[*]**

[#]$p=0.067$ and [*]$p<0.001$

Conclusions: Correlation with the Mitoscore data demonstrated that <20 provided the best overall pregnancy rate and that <25 significantly predicted pregnancy success ($p<0.001$). This analysis confirmed that lower Mitoscore values can be used as biomarkers for embryo quality and can serve as a complimentary selection tool for achieving higher clinical pregnancy rates.

Evaluation of Cryogenic Dewar Health Using a Weight-based Monitoring System

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Objective: To evaluate a weight-based system to monitor changes in liquid nitrogen (LN_2) evaporation rate over time as a method to continuously monitor the overall health of cryogenic dewars.

Design: Prospective experimental study performed within an academic institution.

Materials and Methods: Two cryogenic dewars were used in this study - a new (< 1 yr.) Worthington 35VHC (Dewar A) and an old (> 20 yrs.) MVE – XC 47/11 (Dewar B). Dewars were placed atop CPWplus 75M (Adam Equipment®) scales and weight was continuously recorded under both Control and Experimental conditions using an interface in conjunction with a Network Telemetry Monitoring System (Networked Robotics). Under Control conditions (baseline), dewars were filled with LN_2 and, except for measuring the LN_2 level 3x/week, the dewars were otherwise left undisturbed during the monitoring period. Experimental conditions were identical to the Control, except the dewar's foam core access plug was removed and left off during the monitoring period. One run was performed for each dewar under each condition.

Results: Under both Control (baseline) and Experimental conditions the change in weight of both dewars exhibited a linear relationship with time ($R^2 \geq 0.99$), while the temperature within the dewars remained relatively constant throughout the monitoring period. Linear regression analysis of the Control condition data revealed an LN_2 evaporation rate of 0.340 liters LN_2/day and 0.414 liters LN_2/day for Dewar A and Dewar B, respectively. Similarly, linear regression analysis of the Experimental condition data revealed an LN_2 evaporation rate of 0.954 liters LN_2/day and 1.38 liters LN_2/day for Dewar A and Dewar B, respectively. Differences between the Control and Experiment LN_2 evaporation rates for both dewars could be detected within a few hours.

Conclusions: This study suggests that a weight-based monitoring system could be used to detect relatively small changes in LN_2 evaporation rates under these experimental conditions. The ability to continuously monitor the health of cryogenic dewars via evaporation rate may provide an early detection system for impending cryogenic dewar failure and therefore potentially prevent a catastrophic event. Additionally, the use of a continuously monitored weight-based system could reduce the time, energy, and financial resources involved when manually measuring LN_2 levels 3x/week.

Disclosures: Nothing to disclose.

Funding: REI Division/ UW-Madison School of Medicine and Public Health/ Department of Obstetrics and Gynecology.

Granulosa Cell Mitochondrial Substrate Metabolism Rates Differ between Older and Younger Patients

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Objective: To determine if mitochondrial function in pooled human granulosa cells (GC) differed between older and younger patients.

Design: Prospective study in a hospital based assisted reproductive program and public university. The study assessed the rates of metabolic substrate metabolism by mitochondria in pooled GC from 14 women undergoing in vitro fertilization during 2018 and 2019.

Materials and methods: Both mural and cumulus GC were collected, pooled, and purified from blood cells for each patient. Permeabilized cells were assayed in triplicate for mitochondrial activity using 96-well MitoPlate S-1 plates (Biolog). The plates contained 31 cytoplasmic and mitochondrial metabolic substrates. Metabolism of substrates was assessed by colorimetric change of a terminal electron acceptor tetrazolium redox dye at a wavelength of 590 nm read every 2.5 minutes for 50 cycles on a kinetic plate reader. A metabolic score was created based on the rates of all substrates and normalized against the mtDNA content in each sample. The score was related to patient demographics including age, body mass index, percent of mature oocytes, fertilization rate, day 3 embryo development, blastocyst development, mitochondrial content, and mRNA levels of selected genes. RNA and DNA were isolated from each patient's pooled GCs. Quantitative PCR was performed. The mRNAs evaluated included: amphiregulin (*AREG*), aromatase (*CYP19A1*), hydroxy-delta-5- steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1 (*HSD3B1*), inhibitor of growth protein 1(*ING1*), luteinizing hormone/choriogonadotropin receptor (*LHCGR*), pappalysin-1 (*PAPPA*), TATA-box binding protein (*TBP*). Mitochondrially encoded ATP synthase membrane subunit 8 (*MT-ATP8*) and actin beta (*ACTB*) were quantified from DNA.

Results: Linear regression analysis showed mtDNA content had an inverse relationship with patient age and GC *CYP19A1* mRNA levels ($p<0.05$). Samples were divided into two age groups, < 35 years old and ≥ 35 years old. GC *PAPPA* mRNA levels were significantly increased in the older age group ($p=0.028$). The average rates of metabolism of several substrates significantly decreased in the older age group including: D-gluconate-6-phosphate; D,L-α-glycerol phosphate; L-(+)-lactic acid; L-glutamine; alanine/glycine; α-ketoglutaric acid; α-ketobutyric acid; and palmitoyl-D,L-carnitine with L-malic acid ($p <0.05$). No other parameters showed significant differences.

Conclusions: The average rates of mitochondrial metabolic function for certain substrates decreased in older patients when compared to younger patients. Additionally, pooled GC *PAPPA* and *CYP19A1*mRNA levels were altered between younger and older patients.

Disclosures: Nothing to disclose

Funding: Supported by an ASPIRE-I grant from the University of South Carolina.

High DNA Stainability in the SCSA® is Associated with Poor Embryo Development and Lower Implantation Rate

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Objective: To determine if the high DNA stainability index (HDS), as measured by the sperm chromatin structure assay (SCSA®), is associated with *in vitro* embryo development and concurrent assisted reproductive technology (ART) outcomes.

Design: A retrospective IRB approved, chart review of patients who completed at least one ART cycle (*in vitro* fertilization or intracytoplasmic sperm injection) and had a male partner in a DNA fragmentation index (DFI) of <25% and an HDS level >25%.

Materials and Methods: Semen samples for ART patients were analyzed at SCSA® Diagnostics Inc. A list of male partners with a DFI less than 25% and an HDS greater than 25% was compared to those who completed ART cycle(s) with their female partner. We collected data on the number of patients, the number of cycles, quality of Day 3 and Days5/6 embryos, positive serum human chorionic gonadotropin (hCG) results, fetal cardiac activity (FCA) and live births (LB). We stratified the data into four groups by mean HDS.

Results: 75 IVF cycles had confirmed DFI levels below 25% and HDS levels above 25%. The number of top quality Day 3 and Day 5 embryos decreased when the HDS was above 31% (25%-30%, n=344; 31-35%, n=132; 36-40%, n=79%; 41+%, n=65), however the total percentage of cycles with successful blastocyst formation was similar (25-30% = 33%, 31-35% = 25%; 36-40% = 39%; 41+% = 42%). The total number as well as percentage of positive hCG results after fresh embryo transfer diminished as HDS climbed (25-30%, n = 18 (42%); 31-35% n = 6 (46%); 36-40% n = 4 (44%); 41+% n = 1 (10%). The same trend occurred with FCA (25-30%, n = 18 (42%); 31-35% n = 6 (46%); 36-40% n = 2 (22%); 41+% n = 0 (0%), and LB outcomes (25-30%, n = 14 (33%); 31-35% n = 3 (23%); 36-40% n = 2 (22%); 41+% n = 0 (0%). The implantation rate declined when the HDS was above 36% (25-30% = 30%, 31-35% = 30%; 36-40% = 16%; 41+% = 15%).

Conclusions: These data suggest that while a sperm with an HDS above 25% can fertilize oocytes, as HDS rises, it may be associated with delayed and/or impaired embryo development *in vitro* as well as poor live birth outcomes.

Disclosures: Co-author D. Evenson is the owner and CEO of SCSA® Diagnostics Inc.

Funding: None

Keep or Toss? A Retrospective Evaluation of the Benefits from Culturing 0PN 1PB Embryos Zygotes after ~18hrs Post ICSI

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Objective: We examined embryo development potential and pregnancy outcomes from blastocysts that developed from 0PNpn, 1PB zygotes.

Design: A retrospective study in private IVF laboratory.

Materials and Methods: Over the course of twenty-four months, 68 blastocysts developed from mature oocytes that did not show visual signs of 2 pronuclei at approximently18hrs post-insemination by ICSI. The 0PN1PB embryos were cultured in Single Continuous Culture-NX (Irvine Scientific®) separately from the normally fertilized (2PN) zygote cohort and monitored accordingly. Any blastocysts obtained from 0PN1PB zygotes were either biopsied and/or frozen on day 5, 6 or 7 of embryo development.

Results: January 2017-December 2018, 68 blastocysts obtained from 0PN1PB zygotes represented approximately 1% of the total number of embryos cultured in our laboratory. Of the 68 blastocysts, 51were biopsied and subsequently tested by NexGen sequencing resulting in 23 (45%) of which were deemed euploid. 12 embryo transfers with euploid embryos were performed (11 frozen, 1 fresh) resulting in 5 (42%) with positive clinical pregnancies and 3 live births. Positive clinical pregnancy was defined cardiac activity visualized via ultrasound at 7 weeks gestation. The frequency of chromosomes that were chromosomally abnormal as indicated by PGT-A was also retrospectively evaluated which resulted in no correlation of a particular set of chromosomes

Conclusion: The result of 0PN1PB after ICSI is an artifact of every IVF program. The inefficiency of these embryos to develop into a blastocyst is well known. While the population of embryos produced from 0PN1PBs is considerably low, the minimal extra work and monitoring required does not outweigh the potential benefits of culturing these embryos to the blastocyst stage. Although our study numbers are small, this retrospective observation indicates that these embryos do have the potential to develop into a chromosomally normal blastocyst which can result in a live birth. Embryo transfer data is further limited by preference in selecting embryos which have resulted from 2PN zygotes versus those from 0PN1PB zygotes. However, by utilizing PGT-A technology, our study further validates that these embryos have similar euploid outcomes compared to those blastocysts which have developed from 2PN zygotes. With proper embryo culture organization, these embryos can be monitored effortlessly and become part of any IVF routine.

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Disclosures: None

Repeated High Intensity Laser Biopsy Pulses Do Not Alter Genetic Testing Results nor Increases Mosaicism Following Human Embryo Blastocyst Biopsy of Trophectoderm Cells

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Objective: Recent data suggests that the rate of mosaic diagnosed embryos is increasing following Preimplantation Genetic Testing for Aneuploidy (PGT-A) when using Next Generation Sequencing (NGS). Trophectoderm cell biopsy by laser ablation is proposed as a possible cause of mosaic diagnoses resulting in the subsequent removal of possible genetically normal embryos from availability for embryo transfer. This study investigates whether repeated high intensity laser pulses during trophectoderm laser ablation alters the genetic results or causes increased mosaicism.

Design: Several trophectoderm biopsied cell samples from each embryo were exposed to either repeated laser pulses or increasingly intense pulses prior to NGS analysis for genetic results.

Material and Methods: Embryos were obtained from patients that consented to discarding their surplus embryos. Two genetically undiagnosed blastocysts were used for this study. Each embryo was subjected to low and high intensity laser pulses for varied pulse durations using the Hamilton Thorne Lykos laser. From both embryos 3-5 biopsied cell samples were obtained for each of the laser test categories. Samples were then exposed to direct and repeated laser pulses for 10 or 20 pulses @ 290µs; or 20 or 40 laser pulses @ 400µs. One additional sample, served as the control, and was exposed only to the standard biopsy procedure of 5 pulses @ 290µs. All samples were then washed through several washing buffer droplets prior to placement in PCR tubes in 2-3µl buffer and frozen at -20^{o}C until NGS analysis was performed.

Results: This study demonstrated that genetic analysis results obtained from the control and increased intensity samples show no difference, with all providing normal genetic results (46, XY) (Table1). Importantly, no mosaic genetic results were observed even at the maximum number of pulses or intensity level.

Table 1: NGS Results Obtained from Biopsied Cells Exposed to Repeated Laser Pulses and Increasing Intensity

Embryo	# Laser Pulses	Laser Intensity (µs)	NGS Result
A control	5	290	46, XY
A	10	290	46, XY
A	20	290	46, XY
A	**20**	**400**	**46, XY**
A	**40**	**400**	**46, XY**
B control	5	290	46, XY
B	10	290	46, XY
B	20	290	46, XY
B	**20**	**400**	**46, XY**
B	**40**	**400**	**46, XY**

Conclusion: This study provides significant evidence that multiple laser pulses, even with increased laser intensities, do not alter the genetic results obtained when performing PGT-A. Additionally, the laser intensity does not cause incidences of mosaicism in the NGS results. To minimize potential damage to the embryo and the biopsied cells, optimal excision of the trophectoderm should be achieved using a limited number of laser pulses at the lowest intensity possible. It is important to note, that extreme repeated laser pulses, with increased intensity, may have a detrimental impact on the actual embryo which could reduce implantation potential of the embryo itself.

Thrice Vitrified and Warmed, Twice Biopsied Embryo Originating from a Cohort of Unfertilized Eggs Resulted in Live Birth: A Case Report

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Objective: To report a unique case of an embryo frozen, warmed, and biopsied more than once resulting in live birth.

Design: Case report.

Materials and Methods: A 35 year old nulligravid patient underwent an in vitro fertilization cycle. Her protocol included letrozole 2.5mg days 1-5, recombinant follicle stimulating hormone (FSH: Gonal F) 300 IU and menotropin (Menopur) 75 IU days 1-10. GnRH antagonist (Ganirelix) was administered days 6 - 9. On stimulation day 10, the patient had > 20 follicles ≥ 15mm and estradiol of 8357 pg/mL. A Lupron trigger was given (80 units 35 hours and then 23 hours prior to retrieval). Oocyte retrieval was performed and 52 cumulus oocyte complexes were retrieved. Intracytoplasmic sperm injection (ICSI) was performed on 38 mature oocytes resulting in 17 fertilized oocytes at 17.5 hours post insemination. Due to the low fertilization rate, the unfertilized eggs were observed over the next five days. Two of the eggs thought to be unfertilized, due to the lack of pronuclei the day after ICSI, developed into blastocysts and were frozen with 10 others from the fertilized cohort.

Results: The patient underwent four unsuccessful frozen embryo transfers (FET) with a total of 7 embryos. The five remaining blastocysts were thawed, biopsied, and re-vitrified to perform embryo biopsy and preimplantation genetic testing (PGT) using next generation sequencing. PGT results showed one embryo was euploid (46XY), three were aneuploid, and one, which emerged from the cohort of unfertilized eggs, showed no result. The no-result embryo was thawed a second time, biopsied a second time, and vitrified a third time and repeat PGT showed the embryo was euploid (46XX). This embryo was thawed (for the third time) and transferred in the patient's fifth FET. Beta hCG drawn 9 days post FET was 79 IU/L. A singleton intrauterine pregnancy with fetal heart beat was observed at 6 weeks and 3 days gestation. Delivery occurred at 30 weeks 5 days due to the patient's severe pre-eclampsia. The baby girl weighed 1.5kg and stayed in the NICU for 6.5 weeks to allow for further growth.

Conclusion: Vitrification allows high quality blastocysts to be warmed and re-vitrified without deleterious effects. Previous studies have demonstrated the ability of thrice vitrified/warmed embryos to result in pregnancy as well as twice vitrified/warmed, once biopsied embryos. To our knowledge, this is the first live birth resulting from a thrice vitrified/warmed, twice biopsied embryo resulting from an unfertilized cohort.

Disclosures: Nothing to disclose

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Workflow Patterns Effect pH Variance in the Microdroplet Culture System

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Objective: Determine if disruptive incubator work patterns have prolonged observable effects on pH value variability in culture microdroplets.

Design: This is a quantitative study evaluating real-time pH value variation during incubator disturbance conditions.

Materials and methods: Real time pH data was evaluated under two workflow patterns: low disturbance (incubator opened at beginning/end of the working day for 15 seconds) and high disturbance (incubator opened every hour for 15 seconds). A membrane impregnated with a fluorescent dye-protein conjugate that emits characteristic wavelength spectra at different pH levels was placed at the bottom of a cell culture dish. 50 µL of culture media was placed directly on the membrane under oil and allowed to equilibrate for 24 hours at 37°C and 5% CO_2. pH values were collected from the microdroplet every 30 minutes using a continuous pH monitoring device (SAFE Sens® TrakStation® pH monitoring technology) inside a large format incubator.

Results: The average pH value in the microdroplet before testing was 7.32±0.002 (n = 32) for low disturbance testing and 7.33±0.005 (n = 32) for high disturbance testing. The pH coefficient of variance during high disturbance testing (0.066) was not significant compared to the pH coefficient of variance (0.060) of low disturbance testing.

The average pH value collected during low disturbance testing was 7.32±0.003 (n = 32), and the average pH value collected during high disturbance testing was 7.35±0.013 (n = 32). The pH coefficient of variance during high disturbance testing was 0.182 and the coefficient of variance during low disturbance testing was 0.067.

pH values collected 8 hours after disturbance averaged to 7.32±0.002 (n = 32) for low disturbance testing, and 7.34±0.013 (n = 32) for high disturbance testing. The pH coefficient of variance during high disturbance testing was 0.135 and the coefficient of variance was 0.076 for low disturbance.

Conclusions: Previous pH studies have used indirect measurement techniques that are not representative of the true pH of culture media, as pH can vary due to differences in media volume, oil overlay usage, and geometric shape. Using direct microdroplet pH testing, frequent disturbances in the incubator environment have an observable effect on microdrop culture dish pH variability. The act of opening and closing the incubator door also affects pH variability for at least 8 hours after ending incubator disturbances.

Disclosures: Author's company sells and markets SAFE Sens products.

Funding: None

Are you "Kidding?" A case of Lupus with Autoanti-Jk^a

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Introduction/Background: We describe the case of a 23-year-old African American female who presented to a hospital in the Southeastern USA with chest discomfort and anemia (Hgb 6.3 g/dL). She had a history of recently diagnosed systemic lupus erythematosus (SLE), with no history of pregnancy or transfusion. On admission, she had a pericardial effusion and acute renal failure, and was found to be significantly malnourished. The antibody screen and direct antiglobulin test (DAT) performed at the hospital were both positive. A pre-transfusion sample was sent to our immunohematology reference laboratory (IRL) for investigation.

Materials and Methods: Antibody identification and DATs were performed using standard tube and gel methods. Acid eluates prepared using Gamma ELU-KIT II (Immucor) were tested by gel-IAT. PeG adsorptions were performed using W.A.R.M. treated autologous red blood cells (RBCs). Molecular genotyping was completed using Bioarray HEA Beadchip (Immucor), and confirmed through bidirectional sequencing.

Results: The patient's plasma was visually unremarkable. The DAT-IgG was positive by tube (1+) and gel (3+), and the DAT-C3 was negative (tube). The eluate was pan-reactive. The neat plasma exhibited Jka specificity with dosage, and the autoadsorbed plasma was non-reactive. The patient typed Jka positive by serologic and genomic methods, and sequencing confirmed homozygosity for the Jka-specific polymorphism in *SLC14A1*. No units were ordered at the time of testing, and the hospital ultimately transfused Jk(a-b+) RBCs from their inventory.

Conclusions: These results are consistent with an autoanti-Jk^a. This autoantibody is observed only rarely, most commonly in patients with autoimmune anemia stemming from either a broader autoimmune condition or response to certain drugs. Our patient's medication list included none of the implicated drugs but with the history of SLE, our data support the association of autoanti-Jka and autoimmune conditions. No testing (e.g. LDH/Bilirubin) was performed by the hospital to determine if immune hemolysis was occurring in this case, and there were other potential reasons for the anemia.

Transfusion decisions must weigh the risk from autoantibody against the risk of forming an alloantibody. Although Jk^b is a relatively poor immune stimulator, Jk^b negative patients transfused with Jk(a-b+) blood are at risk of forming alloanti-Jk^b, and finding Jk(a-b-) donors is exceedingly difficult (frequency <0.01%). In absence of proven clinical significance for the autoantibody, clinical signs of hemolysis, or significantly reduced RBC survival, selection of Jk(a+b-) red cells may be preferred in similar cases.

Disclosures: None.

Funding: None.

The Benefits of Performing Laser Hatching at the Time of Trophectoderm Biopsy

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Objective: To describe the multiple benefits of performing laser hatching for trophectoderm (TE) biopsy at the time of biopsy rather than at an earlier embryonic stage.

Type of Study: Case Report/Opinion

Materials and Methods: Embryos were cultured in Global Total Culture Media (Cooper) and were not evaluated after the fertilization check until Day 5 of development. Blastocysts (D5/D6) were biopsied using a Hamilton Thorne Zilos laser on an Olympus IX 71 Inverted microscope. Biopsied blastocysts were vitrified on Cryotops using Kitazato Vitrification kits (CA Cryobank).

Results: Blastocysts were placed in 6ul mHTF drops under oil and were picked up with holding pipette with the inner cell mass (ICM) placed between 7 and 10 o'clock. Blastocysts were collapsed with a 250µm laser pulse at 3 o'clock and biopsy did not occur until the blastocyst was totally collapsed. The laser was increased to 450µm and the biopsy pipette was introduced into the small hole that was made during collapse. With suction on the biopsy pipette, approximately 5-8 trophectoderm cells were pulled toward the biopsy hole, which stretches the cells and several pulses of the laser sheared the cells off into the biopsy pipette. The biopsy sample was located in the drop and the blastocyst was returned to a labeled post-biopsy holding dish. The blastocysts were vitrified after the biopsied cells were loaded into microfuge tubes and frozen.

Conclusions: When trophectoderm biopsy became the preferred method for biopsy, most IVF labs performed laser hatching on Day 3 of embryo development followed by a Day 5/6 biopsy. Often the ICM hatched out of the hole that was created. In addition, many of the blastocysts had very thick zona pellucidas (ZP) and some had very low TE numbers. By performing laser hatching at the time of biopsy, the ZP is normally very thin and the TE number is what it should be for a Day 5/6 blastocyst. By not breaching the ZP on Day 3 the embryo develops into an expanded blastocyst like the embryos in culture that are not undergoing biopsy. Usually, 6-8 TE cells are removed during the biopsy procedure and the collapsed blastocyst tolerates the procedure without any visble negative effects. The collapsed blastocyst vitrifies very well and expands quickly post-thaw.

Disclosures: Nothing to disclose

Funding: None

Blastocyst Formation and Pregnancy Rate Comparison Between Standard Large Box (Sanyo) Incubators and a Novel Geri® Time-Lapse Incubator.

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Objective: We compared the blastulation rates and pregnancy success from embryos cultured in standard "large box" Sanyo incubators and the Geri® time-lapse incubator.

Design: A retrospective study in a private IVF laboratory.

Materials and Methods: Mature oocytes were fertilized by either conventional insemination or ICSI and cultured to the blastocyst stage undisturbed in Continuous Single Culture®-NX (Irvine Scientific) at 37°C in 6% CO_2. Patients could self-elect to be included in the Geri incubator prior to retrieval. Availability for the Geri was limited to six culture chambers. Patients with a minimum of one mature oocyte with normal fertilization were eligible to have their embryos cultured in the Geri. All embryos were either biopsied and/or frozen on Day 5, 6 or 7 of development.

Results: The evaluation was performed over a six-month period (August 2018-February 2019). A total of 67 patients had their embryos cultured in the Geri. A total of 611 zygotes were cultured to the blastocyst stage, resulting in 247 blastocysts – a 40.4% blastulation rate. In comparison, 288 patients yielded 2,387 zygotes that were cultured in the Sanyo incubators, resulting in 1,055 blastocysts a 44.2% blastulation rate. Chi-square analysis showed no significant difference in blastulation rates ($p=0.0932$) between incubator types. In addition, 279 frozen embryo transfers (16 Geri and 264 control) were performed with no significant difference ($p=0.9556$) in pregnancy success, 33% and 34%, respectively. Pregnancy success was identified by positive fetal cardiac activity at seven weeks gestation. Time-lapse morphokinetic assessment was not applied during this evaluation period.

Conclusions: In the IVF laboratory, several types of incubators are available, and each requires careful consideration. The Geri, an independent chamber/microscope incubator, offers a safe environment for embryo culture with minimal disturbance. Blastulation rates did not differ when compared to the large box incubators, allowing for another option for embryo culture. The Geri has six chambers, which can accommodate a maximum of 16 embryos per Geri Dish, which may be limiting factor to some laboratories. However, the Geri Dish is unique in that it allows for potential benefits of group culture while maintaining individual embryo growth tracking. The ability to assess the embryos in real time does provide opportunities for clinical applications for embryo selection. We are continuing to monitor pregnancies and embryo culture outcomes accordingly.

Disclosures: EMD Serono provided the incubator at no cost during the embryo evaluation period.

Funding: None

A Case of Recurrent Spontaneous Parthenogenetic Oocyte Activation:

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Oocytes obtained during in vitro fertilization (IVF) cycles ideally are arrested in metaphase II (MII), which are characterized by the presence of a single polar body. They remain in arrest until after fertilization when time meiosis is resumed in a calcium-dependent manner.

We present a case of a 23 year old gravida 0 with polycystic ovarian syndrome who presented for evaluation at our center after previously having failed two IVF cycles elsewhere. The patient subsequently underwent three controlled ovarian hyperstimulation cycles with either an antagonist or low dose luteal lupron down-regulation protocol. Vaginal oocyte retrieval (VOR) was performed 36 hours after administration of gonadotropin releasing hormone agonist (GnRHa) or human chorionic gonadotropin trigger. Cycle outcomes are demonstrated in Figure 1. Oocytes were fertilized by intracytoplasmic sperm injection (ICSI) according to standard laboratory protocol. Embryos were cultured until day 7 of development. A substantial proportion of oocytes were noted to have one pronucleus (1PN) and two polar bodies (PB) at the time that the cumulus complex was denuded prior to ICSI (Figure 2). The parthenogenetic oocytes were re-examined on the morning following VOR and were noted to have undergone cell division, with many that appeared similarly to cleavage stage embryos. In each cycle, there was complete embryo arrest prior to the blastocyst stage and no embryos were available for transfer.

This case of recurrent parthenogenesis, accelerated cell division and complete developmental arrest highlights what potentially could be a rare genetic contribution to a cell-cycle regulatory deficiency. It has been suggested that defects in the c-mos/mitogen-activated protein kinase (MAPK) pathway in oocytes exhibit failure to arrest and premature activation (1). EMI2 is an additional regulatory factor that has been implicated in maintenance of arrest (2). It is unclear whether there is a failure of MII arrest due to functional deficiencies of MAPK and EMI2 or if there is an unidentified stimulus for escape which overrides these checkpoints. Attempts at fertilization of prematurely activated 1PNs have previously failed (3). Enhanced understanding of cell-cycle regulatory measures in oocytes and human preimplantation embryos may yield insight regarding diagnostic and therapeutic modalities for similar rare cases of spontaneous parthenogenesis.

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Figure 1.

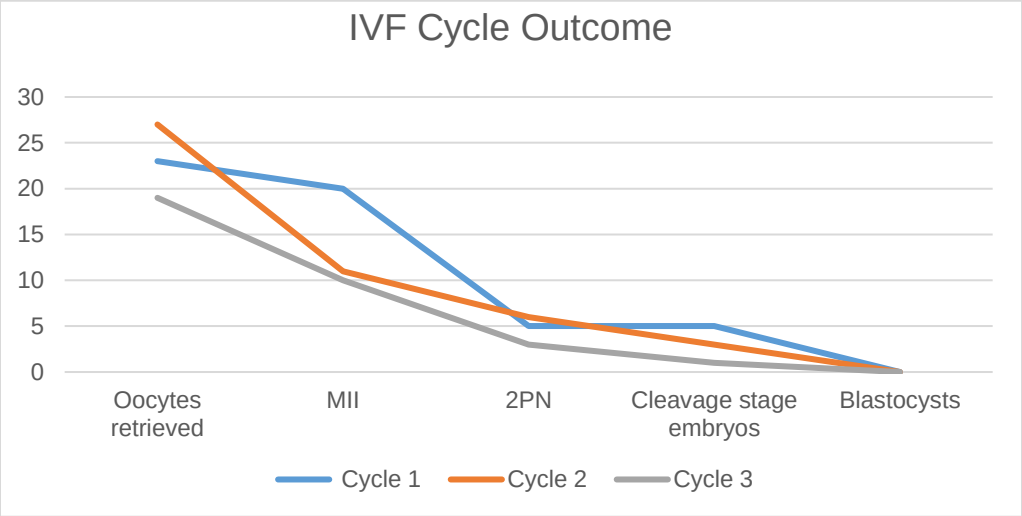
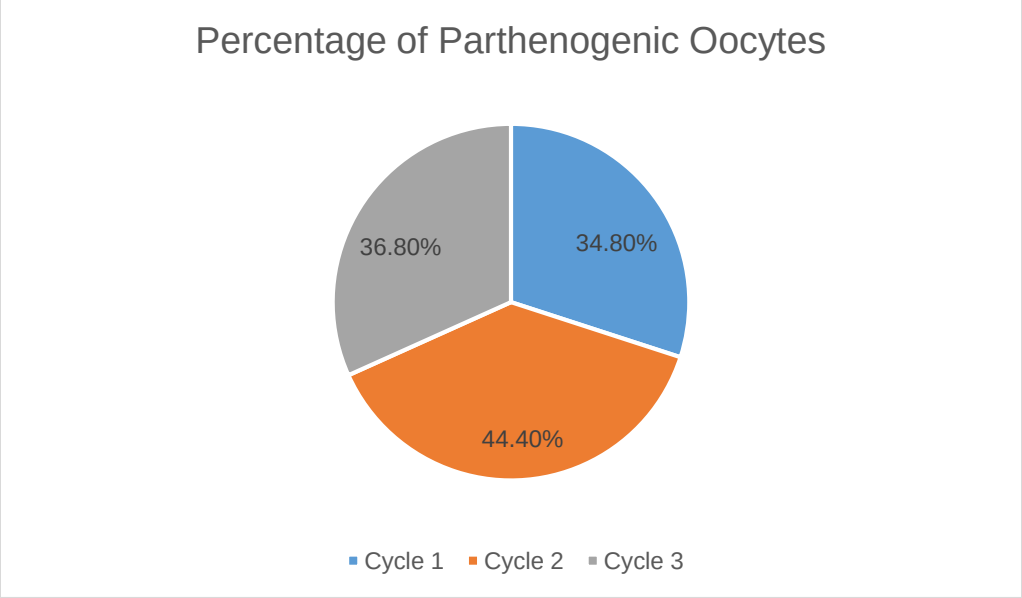


Figure 2.



Continuous Monitoring of Incubator and Cryotank Parameters

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Objectives: 1. To continuously monitor temperature, humidity and carbon dioxide (CO_2) levels in a CO_2 incubator during daily use, and 2. To correlate temperature and weight of embryo storage dewars over time.

Design: Continuous monitoring in an embryology laboratory.

Materials and Methods: 1. HeraCell 150i temperature was set to 37.0ºC, and CO_2 to 6.5%. Humidity was generated passively using a water pan. Wired temperature, CO_2 and humidity monitors were installed on the right side of the middle shelf. 2. LN_2 levels were monitored daily in MVE cryotanks using a yardstick while dewar weights were monitored by wired probes placed on the outside of the dewars. Data was collected every 5 sec from the wired monitors over the course of several months. Data for all parameters was viewable on the website and could be printed and graphed for each parameter.

Results: 1. During working hours mean temperature was significantly lower compared to non-working hours (36.9 ± 0.19 ˚C vs. 37.1 ± 0.09˚C; P<0.001). Working hour mean CO_2 was also lower compared to nonworking hours (6.19 ± 0.20% vs. 6.33 ± 0.30% P<0.001). Finally, working hour humidity was also significantly less compared to that recorded during non-working hours (92.5 ± 0.64% vs 94.66 ± 0.20%; P<0.001). All parameters decreased immediately upon first opening of one of the three small interior incubator doors and did not return to nonworking hour levels until approximately 2 hours after the last opening for temperature, 4 hours for CO_2, and 7 hours for humidity. 2. Dewar weight changes monitored electronically decreased proportionally to measured LN_2 levels.

Conclusions: 1. Even a single opening of one of the three small doors in the incubator was sufficient to bring about a significant fluctuation in temperature, CO_2 and humidity and none of the parameters stabilized to pre-opening levels throughout the day. These changes in incubator parameters may result in pH drift in media throughout an 8 hr period that may have significant consequences if incubators like these are being used for embryo culture for several days. 2. Remote cryotank weight monitoring can provide a second verification of tank status. Continuous measuring and data recording of incubator parameters and cryotank weight are crucial to fine-tuning service and security for IVF patients.

Disclosures: Probes and IOT devices provided by TekConscious.

Funding: None

Does Culture of Embryos in an Ultra-Low (2%) Oxygen Environment Yield Better Blastocyst Development than 6% Oxygen Using Time-Lapse Morphokinetics?

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Objective: To evaluate if culture of mouse embryos in an ultra-low oxygen environment could enhance blastocyst development in terms of time-lapse morphokinetics.

Design: Prospective study

Materials and methods: A total of 214 commercially obtained frozen mouse embryos (B6D2F1 & B6C3F1 hybrid) were thawed and cultured in One-Step medium with 10%SPS using an EmbryoScope time lapse incubator at 37°C, 5.5% CO_2, and either (1) 6.0% O_2 (n=106) or (2) 2.0% O_2 (n=108) Embryo images were recorded every 10 minutes for 6 days of culture. Time lapse videos were annotated for the following time points: 2cell (t2), 3cell (t3), 4cell (t4), 5cell (t5), 6cell (t6), 7cell (t7), 8cell (t8), start of compaction (tSC), morula (tsM), start of blastulation (tSB), blastocyst (tB), expanded blastocyst (tEB) and hatching blastocyst (tHB). The 2-cell stage was considered as time zero since the exact time of insemination was unknown. Time points were statistically compared between the two groups. Blastocyst development rates for both culture environments were also compared.

Results: There were no statistically significant differences between the two groups in any of the time points measured up to the 8-cell stage. However, after the 8-cell stage, the 2% O_2 group showed significantly slower embryo development for each time point up to the hatching blastocyst stage. There was no difference in the blastocyst development rate between the 6% O_2 and 2% O_2 environments (99.1% vs 95.4%).

Oxygen level	Time points (hrs)												
	t2	t3	t4	t5	t6	t7	t8	tSC	tM	tSB	tB	tEB	tHB
6% O_2 (n=106)	0.0 ±0.0	19.7 ±2.4	21.0 ±2.7	29.5 ±1.9	29.9 ±2.0	30.9 ±2.5	30.9 ±2.1	35.2[a] ±2.9	41.5[b] ±3.7	56.7[c] ±3.8	63.3[d] ±5.9	69.7[e] ±7.6	79.7[f] ±8.6
2% O_2 (n=108)	0.0 ±0.0	20.0 ±1.8	21.0 ±1.7	29.4 ±1.6	29.8 ±2.0	30.9 ±2.4	31.6 ±2.9	36.1[a] ±3.1	42.9[b] ±3.3	59.3[c] ±5.9	68.6[d] ±7.7	77.6[e] ±9.8	86.0[f] ±8.6

[a,b,c,d,e,f]*P<0.05. Time points are expressed as Mean ± SD.*

Conclusions: Our data did not show any benefit of culturing embryos in a 2% oxygen environment. Although there was no difference in the blastocyst development rate between culturing in 2% and 6% oxygen, the 2% oxygen group took significantly longer to reach the blastocyst stage than the 6% oxygen group.

Disclosures: Nothing to disclose

Funding: None

Embyros With No Initial PGT-A Result Can Undergo Warming/Rebiopsy/Revitrification For An Attempted Reanalysis, However They Ultimately Demonstrate Very Low Clinical Potential

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Background: PGT-A with Next Generation Sequencing (NGS) is becoming the standard of care, and the number of blastocysts biopsied is increasing. However, there are a small percentage of blastocysts where a result is not obtained from the initial genetic analysis. The immediate desire is to often proceed with reanalysis by warming/rebiopsy/revitrification (WrBrV) for repeat PGT-A analysis. We investigated the efficacy of subjecting previously biopsied blastocysts to WrBrV in a second attempt to obtain genetic results.

Objective: To determine whether it is laboratory efficient to attempt WrBrV in order to obtain a PGT-A result from a previously biopsied blastocysts.

Materials and Methods: Previously biopsied and vitrified blastocysts were warmed using our standard protocol (Irvine Scientific Thaw). Assessment of blastocoel cavity expansion was performed ~2 hours post warming; followed by trophectoderm rebiopsy, where an additional 3-5 cells were excised. The biopsied cells were placed in PCR tubes for analysis and the blastocysts were immediately revitrified.

Results: From January 2018 through February 2019, PGT-A was performed on trophectoderm cells from 2715 embryos. A 'No result' was obtained from 33 embryos (1.2%); and all were Good and Fair quality Hatching Blastocyst (HB-G, n=5; HB-F, n=28). Physicians ordered WrBrV for only 12 blastocysts (36%). Two embryos degenerated following warming and were not rebiopsied (17%). The remaining 10 blastocysts underwent biopsy and genetic results were obtained from all. Only two blastocysts were diagnosed as euploid (20%); and a Frozen Embryo Transfer (FET) was performed with a single euploid blastocyst, but resulted in a failed implantation.

Conclusions: At our center, FETs performed with single euploid blastocysts result in a 69% pregnancy rate. This demonstrates that the biopsy, vitrification and warming techniques as performed by the embryologists at our center do not significantly diminish the potential of euploid embryos to implant. Following an initial PGT-A with no NGS result (1.2%), the process of WrBrV in our laboratory has resulted in an 83% survival rate; however only 20% of embryos have been euploid and 0% have implanted following FET, demonstrating the clinical significance and laboratory efficiency is extremely low. This study, although small in size and in need of further investigation, supports that embryos with no initial NGS result, although of good/fair quality and available for WrBrV, have a very high incidence of aneuploidy and may not warrant additional laboratory resources and reanalysis; especially when other euploid embryos are available.

Finding a Better Mouse Model for Quality Control and Research Studies in the IVF Laboratory

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Objective: Since the strain of mouse embryos routinely used for quality control in IVF laboratories almost always have >90% blastocyst development, possibly due to improved culture conditions, it is very difficult to detect negative effects of media that would that would otherwise affect human embryo development. The aim of this study was to find a strain of mouse embryos that would be more sensitive to culture changes for use as a marker for quality control as well as for research studies.

Design: Prospective study

Materials and methods: One-cell embryos from B6D2F1 & B6C3F1 Hybrid mice (Strain 1) and from C57BL-6N mice (Strain 2) were used for this study. Embryos were thawed and cultured in different types of media: (1) One-Step medium, (2) One-Step medium with IGF-1, and (3) One Step medium with insulin. All embryos were cultured in an EmbryoScope incubator at 37°C, 5.5% CO_2 and 6% O_2 for 6 days. The blastocyst formation rate was calculated as the number of blastocysts per embryos cultured for each group and compared statistically using a Chi-squared test.

Results: The data showed that Strain 2 embryos had significantly lower blastocyst development rates than Strain 1 embryos in One-Step medium routinely used in IVF culture (Table 1). Furthermore, when testing the effect of adding IGF-1 or insulin to the media, Strain 2 embryos showed more pronounced changes in blastocyst development rates compared to Strain 1. The insulin supplemented media resulted in significantly lower blastocyst development rate for both strains.

Table 1: Blastocyst development rates between two strains on mouse embryos cultured in One-step, IGF-1 and Insulin.

Culture media	Strain 1 (B6D2F1 & B6C3F1 Hybrid)	Strain 2 (C57BL-6N)	p-value
One-Step (n=238)	99.1% (n=106) [a]	80.3% (n=132)[b]	*P<0.0001*
IGF-1 (n=249)	96.2% (n=105)	74.3% (n=144)	*P<0.0001*
Insulin (n=249)	93.3% (n=105)[a]	68.8% (n=144)[b]	*P<0.0001*
p-value	*[a]P=0.02*	*[b]P=0.02*	

Conclusions: The C57BL-6N strain of mouse embryos appears to be a better model for detecting subtle changes in culture conditions since it is more sensitive than the B6D2F1 & B6C3F1 Hybrid strain. Ongoing studies are aimed at assessing if there are any changes associated with time-lapse morphokinetics.

Disclosures: Nothing to disclose

Funding: None

Increased Expansion and Decreased Contraction of Embryos Corresponds to Increased Clinical Pregnancy Rates in Single FET Cycles

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Objective: To investigate the possible association between expansion or contraction of blastocoeles after thawing with ongoing clinical pregnancy rates in frozen embryo transfer (FET) cycles. We hypothesize a greater change in blastocele size between thaw and transfer will correspond with an increase in pregnancy rate.

Design: Retrospective Study

Materials and Methods: A total of 257 single FET cycles from November 2017 to January 2019 were included. Patient ages ranged from 24 to 48 years. Programmed FET priming was performed with exogenous estrogen and progesterone. 67 embryos that underwent PGT-A (Preimplantation-Genetic Testing for aneuploidy) and 9 donor egg cycles were included. All blastocysts were cultured in trigas incubators ranging from 20 minutes to 4 hours 42 minutes. Pictures of each blastocyst were taken utilizing the Hamilton Thorne Zilos laser software (Beverly, MA) immediately after thaw and before transfer. The longest portion of the embryo was measured in µm using the ruler tool on the laser by one of two laboratory technicians. Pregnancy was defined by a positive HCG 9 days after embryo transfer and ongoing clinical pregnancy was defined by the presence of fetal cardiac activity at 6 ½ weeks. Wilcoxon rank sum tests were used to access differences in change parameters.

Results: Using the two measurements and their time difference we analyzed the amount of expansion and contraction and the rate of expansion and contraction. The 186 women that had embryos that expanded between thaw and transfer were more likely to have ongoing pregnancies than those whose embryos did not expand (median expansion 27.0µm vs. 19.6µm, p=0.036). The median percent change of expansion was also significantly different between groups (p=0.028) (Table 1). Among the 70 embryos that contracted, there was a trend between more distance contracted and lower ongoing pregnancy (p=0.075) and percent change of contraction and ongoing pregnancy (p=0.058), but neither reached significant.

Table 1. Expansion and Contraction of Embryo by Clinical Pregnancy [Median (Range)]

	Cohort	Ongoing Pregnancy		
		Not Pregnant	Ongoing Pregnancy	p-value
Total Number of Patients	N = 257	N = 128	N = 129	
Age (±SD)	34.3 (±4.7)	34.7 (±4.9)	34.0 (±4.4)	0.244
PGTa (%N)	67 (26%)	29 (23%)	38 (29%)	0.214
Time Difference (minutes)	112 (20 - 282)	106 (20 - 242)	116 (30 - 282)	0.055
Expansion (μm)	24 (0 - 147.3)	19.6 (0 - 103.6)	27.0 (0.1 - 147.3)	0.036*
Contraction (μm)	-7.5 (-47.7 - -0.1)	-8.2 (-47.7 - -0.1)	-4.5 (-33.5 - -0.5)	0.075
Percent Change Expansion	18.7 (0 - 110.9)	15.8 (0 - 95.4)	21.0 (0.08 - 110.9)	0.028*
Percent Change Contraction	-5.8 (-28.84 - -0.1)	-7.0 (-28.84 - -0.1)	-3.3 (-22.81 - -0.3)	0.058

***Significance indicated at p-value<0.05**

Conclusions: We found a significant increase in ongoing pregnancy rates from embryos with greater expansion rates and percent changes in expansion. We also found a trend between increased pregnancy rates and smaller amounts of embryo contraction and percent change of contraction, however, neither reached significance. According to this study, a larger degree of embryo expansion post thaw has a better chance of ongoing pregnancy. Additional studies are needed to determine if smaller degrees of contraction is suggestive of ongoing pregnancy outcome.

Disclosures: None.

Funding: None.

Insulin and IGF-1 Does Not Alter the Morphokinetics of Mouse Embryo Development

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Objective: To assess if there is a benefit to adding either IGF-1 or insulin to culture medium in terms of mouse embryo development using time-lapse morphokinetics.

Design: Prospective study.

Materials and methods: A total of 305 commercially obtained frozen mouse embryos (B6D2F1 & B6C3F1 hybrid) were thawed and cultured in (1) One-Step medium only, (2) One-Step medium with 100ng/mL insulin, and (3) One-Step medium with 100ng/mL IGF-1, using an EmbryoScope time lapse incubator at 37°C, 5.5% CO_2, and 6.0% O_2. The EmbryoScope was set to record images of each embryo every 10 minutes for 6 days of culture. The following time points were annotated: 2cell (t2), 3cell (t3), 4cell (t4), 5cell (t5), 6cell (t6), 7cell (t7), 8cell (t8), start of compaction (tSC), morula (tsM), start of blastulation (tSB), blastocyst (tB), expanded blastocyst (tEB) and hatching blastocyst (tHB). The 2-cell stage was considered as time zero since the exact time of insemination was unknown. All time points were statistically compared between each of the three groups. Blastocyst development rates for each group were also compared.

Results: A total of 304 blastocysts developed from the 316 embryos cultured, yielding an overall blastocyst development rate of 96.2%.When comparing the blastocyst development rate between the 3 groups, there were no significant differences between the One-Step and IGF-1 media groups (99.1% vs 96.2%). However, the insulin group showed significantly lower blastocyst rates when compared to the controls (93.3% vs 99.1%; p=0.02). There were no statistically significant differences in any of the time points measured between the One-Step, insulin and IGF-1 groups.

Media	Time points (hrs)												
	t2	t3	t4	t5	t6	t7	t8	tSC	tM	tSB	tB	tEB	tHB
One-Step (n=105)	0.0 ±0.0	19.7 ±2.4	21.0 ±2.7	29.5 ±1.9	29.9 ±2.0	30.9 ±2.5	30.9 ±2.1	35.2 ±2.9	41.5 ±3.7	56.7 ±3.8	63.3 ±5.9	69.7 ±7.6	79.7 ±8.6
Insulin (n=99)	0.0 ±0.0	20.0 ±1.7	21.0 ±1.9	29.6 ±1.9	30.1 ±1.8	30.8 ±1.8	31.0 ±2.2	35.2 ±2.3	42.0 ±3.0	57.6 ±3.5	62.9 ±4.3	69.3 ±6.4	80.4 ±8.7
IGF -1 (n=101)	0.0 ±0.0	20.0 ±1.4	20.9 ±2.1	29.7 ±2.0	30.0 ±2.1	30.9 ±2.3	31.0 ±2.3	35.2 ±2.7	41.9 ±3.1	56.7 ±3.7	62.3 ±4.6	68.8 ±6.6	79.6 ±7.5

Time points are expressed as Mean ± SD.

Conclusions: The data indicates that there is no beneficial effect of adding insulin or IGF-1 to culture media for mouse embryo development. Neither insulin not IGF-1 had any effect on the morphokinetics of these embryos. However, culture of mouse embryos in insulin supplemented media resulted in a lower blastocyst development rate compared to One-Step media. Ongoing studies are underway using a more sensitive mouse strain to see whether any changes in morphokinetics may be detected.

Disclosures: Nothing to disclose

Funding: None

Molecular Aspects of Aneuploidy in Preimplantation Human Embryos: A Mini-Review

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Objective: Focus and analyze on the two aneuploidy types (meiotic and mitotic) in preimplantation human embryos, their causes and possible molecular mechanisms involved.

Design: Mini-review.

Materials and Methods: Search performed in MEDLINE database under the keywords; aneuploidy, molecular mechanisms, preimplantation embryos.

Results: Aneuploidy is the major obstacle in order to achieve successful pregnancies in IVF world. Specifically, more than 50% of preimplantation embryos are aneuploid and unable to achieve pregnancies and live births. In reproductive aged women, aneuploidy rate in oocytes, caused by an error-prone meiotic chromosome segregation mechanism, is reaching 20-30%, in contrast to human sperm, that only 1-8% have an abnormal chromosomal content. Meiotic aneuploidy can occur from MI non-disjunction, MII non-disjunction and MI or MII pre-division. Maternal age is the major critical factor related to aneuploidy; 50% of the oocytes from advanced age women ($\geq$ 40 years old) are aneuploid due to meiotic errors. Cohesive chromosome connections are weakened with increasing maternal age mainly due to very small amounts of a major cohesin component, the Rec8. Additionally, recombination failure, which varies between chromosomes, can also lead to meiotic aneuploidy.

Genomic errors can also arise during post-fertilization mitotic divisions, resulting in embryonic mosaicism. The mechanisms leading to embryonic mosaicism during embryonic mitosis are; non-disjunction, anaphase lagging and endoreplication. Mitotic aneuploidies rise from 63% at the cleavage stage to 95% in blastocyst stage human embryos. Defects in mitotic spindle assembly checkpoint (SAC) can result to high chromosome missegregation levels both *in vitro* and *in vivo*. Furthermore, malfunction of cohesins, possibly due to inactivation of STAG2 and/or overexpression of seperase, leads in premature chromosome separation, while delay in their removal may result in non-disjunction. Finally, embryonic mitotic aneuploidy can be caused by extra-nuclear DNA formation (micronucleation) from lagging chromosomes with severe DNA damage and by severe paternal sperm defects.

Conclusions: The high rate of chromosomal aneuploidies may arise mostly during the first or second meiotic divisions, especially in advanced maternal age women, but also can arise in the postzygotic stage during the mitotic divisions of cleavage stage and blastocyst embryos, resulting in mosaicism. Further work and analysis on the molecular mechanisms that lead to meiotic and mitotic aneuploidy in preimplantation embryos, can reveal improved clinical strategies to reduce the occurrence of aneuploidy and consequently improve the success rates of IVF.

Disclosures: None.

Funding: None.

Preimplantation Embryo Ploidy Status Correlates With Morphokinetic Cell Cycle Timing

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Objective: To determine if morphokinetic parameters differ between euploid and aneuploidy emryo development using time-laps imaging (TLI) and preimplantation genetic screening (PGS).

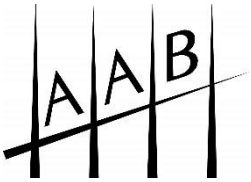
Design: Retrospective chart review

Materials and Methods: A total of 613 embryos from 128 patient couples were evaluated for ploidy status and morphokinetic time points using TLI January ,2016- March,2017 were analyzed for TLI, ploidy status and infertility diagnosis. The subject population included couples going through IVF using TLI culture and PGS testing by NGS or aCGH. Important morphokinetic cell divisions were annotated : 2-9+ cell stages annotated as t2, t3, t4, t5, t6, t7, t8 and t9+ and morula formation), starting blastulation(tSB), expanding blastocyst(tEB), and hatching blastocyst(tHB) determined. The Chi square test was used to detect associations between ploidy status,age,infertility diagnosis and PGS results. Two sample student t test was used to compare the difference in average morphokinetic outcomes, t2-t8, between euploid and aneuploid embryos. Cell cycle time between the 2 and 3 cell stage (CC2) and between the 3 and 4 cell stage (S2) were calculated. As these differences lack the normality assumption , we analyzed the difference between CC2 and S2 by using Wilcoxon-Mann Whitney U test to compare between aneuploidy and euploid PGS results. Diagnosis comparisons displayed as descriptive statistics.

Results: There were no significant differences in most morphokinetic time points between euploid and aneupliod embryos. There was a statistically significant difference between euploid and aneuploid development in the CC2 (-1.088±7.093; p-value= 0.0404) as well as a marginal difference between euploid and aneuploid embryos is S2 (0.963±0.0512; p-value= 0.0512) . It is noticed that ploidy status significantly different between female ≤ 34 yrs. and > 34 yrs. of age (p-value=0.043). The odds of euploid among female ≤ yrs. is 1.465 times compared to that of females > 34 yrs. old. Therefore, younger females are 47% more likely to have euploid embryos compared to older women.

Conclusions: The observation and comparison of kinetic behavior through TLI and morphokinetic calculations of CC2 and S2 can aid in prediction of embryo ploidy status in combination with PGS test results.

References: Chawla M et al. Morphokinetic analysis of cleavage stage embryos and its relationship to aneuploidy in a retrospective time-lapse imaging study. Assist Reprod Genet. 2015;32:69-75.



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