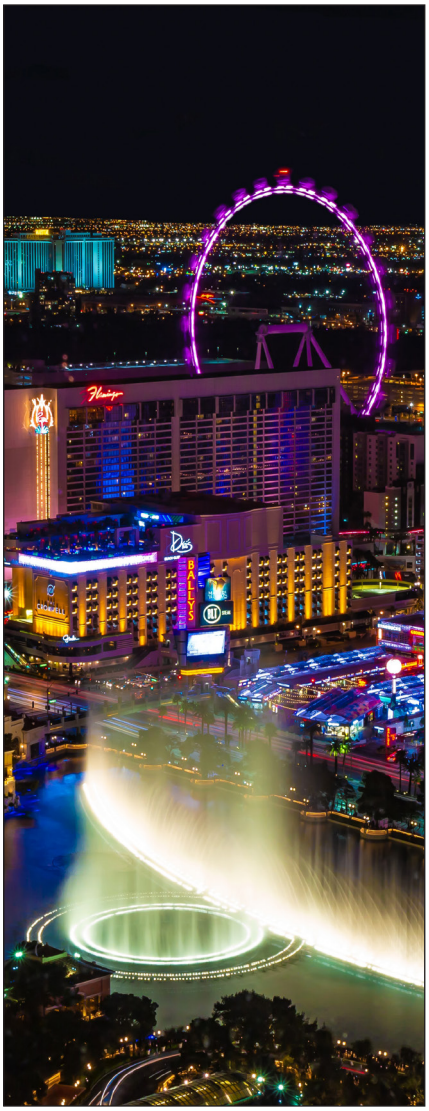
Conference & Symposium

April 29-May 2, 2025

JW Marriott Las Vegas Resort & Spa • Las Vegas, Nevada



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ABSTRACTS – ORAL

ABSTRACTS – POSTER

Direct Rehydration of Vitrified Human Blastocysts

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Abstract

Objectives: To comparatively assess the ability of conventional and ultra-fast vitrified (CV and UFV, respectively) blastocysts to display resilience to the osmotic stress of post-warming rehydration under extreme conditions and to objectively determine their potential viability.

Design: Prospective, apriori arrangement of post-warming treatments comparing 1-step rapid elution (RE) in a non-permeating solute (e.g., sucrose) to direct rehydration (DH) in G-MOPS medium was applied to conventionally vitrified research discard blastocysts and then to UFV re-vitrified blastocysts.

Materials & Methods: 101 research discard embryos vitrified in standard EG/DMSO solutions onto s-Cryolock and Cryotips devices systems were warmed rapidly by manufacturer's protocols. Using pre-warmed (37°C) thawing solutions, embryos were decanted directly into either 1M sucrose or isotonic G-MOPS medium for 1 min. All embryos were washed in isotonic solutions prior to being pipette into LG culture medium + 10% LGPS. A subset of these embryos (n=32) were re-vitrified on Cryo-go devices (Irv. Sci./ Fujifilm) following an ultra-fast exposure protocol (2min ES/ 1min VS; NX vit solution, IS/FF). These embryos were held in LN2 for >30min before rapid warming and repeat post-warming treatments by RE or DH methods. The other 70 embryos remained in culture up to 48h to assess sustained developmental competence (BL expansion, hatching, trophoblastic outgrowth). As an additional assessment of viability, we confirmed the autofluorescence of cell energetics measuring NADH and FAD activity at 700nm of light (i.e., fluorescent lifetime imaging microscopy, FLIM) at +3-5h or +24-28h post-warming.

Results: All DH-treated CV blastocysts (n=51) survived warming similar to RE-treated blastocysts (49/50; 98%). Post-warming survival was equal at 3-5h (92% re-expansion) and at +24h (82% expansion or hatching). The latter observations were further confirmed by the emission of photonic energy visualized by FLIM technology. Additionally, RE-treated (58.3%) and DH-treated (70.8%) embryos both formed trophoblastic outgrowths at +48h. All re-vitrified blastocysts survived post-warming rehydration, independent of treatment, with 87.5% re-expanding at +2h/treatment.

Conclusions: The osmotic neutrality of direct hydration while rapidly warming vitrified embryos defies cryobiologic principles. We anticipated its effectiveness under non-equilibration UFV where a minimal amount of cryoprotective agent (CPA) permeates blastomeres to achieve a vitrified state. However, we were surprised to confirm the relatively equal exchange of water and CPA among CV-treated embryos without cell lysis. The osmotic resiliency, based on high survival rates and sustained viability, of blastocysts to DH proved comparable to standard RE treatment.

Disclosure: None

Funding: None

Key words: vitrification, embryo, dilution, rehydration

The Effect of Buffer Volume on Preimplantation Genetic Testing (PGT) Sample Failure Rates

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Objective: PGT for aneuploidy (PGT-A) sample failure rates can vary between PGT laboratories and IVF laboratories. Various factors may contribute to a sample failing quality control (QC) metrics. On many next generation sequencing (NGS)-based testing platforms, the overall sample quality metric is measured through median absolute pairwise differences (MAPD) score. Higher MAPD scores are correlated with lower read count and a "noisier" profile, with a typical cutoff of 0.3 for sample failure. This study aims to identify any correlation between sample buffer volume and MAPD score.

Design: Retrospective analysis of trophectoderm biopsy samples processed by NGS.

Materials and Methods: Cases received between January and June 2023 were considered for inclusion. Samples were spun down upon receipt and categorized into the following volume ranges: < 2 µL, 2-5 µL, and 5-10 µL. Whole genome amplification, NGS, and data analysis were performed using the Ion ReproSeq™ PGS Kit and Ion Reporter™ software (Thermo Fisher Scientific). Only batches with ≥10 million total reads were included in this study. Once processed, the samples were grouped by MAPD score ranges and chi square analyses were performed.

Results: A total of 1909 trophectoderm biopsy samples from 459 cycles were initially included. Due to very low sample size (n = 9), samples with a 5-10 µL volume range were later excluded. Table 1 summarizes the collected data and analyses.

Table 1: MAPD Score by Buffer Volume

	MAPD Score		
Buffer Volume	**< 0.200**	**0.200 – 0.299**	**≥ 0.300**
< 2 µL	546 (58%)	380 (40.3%)	16 (1.7%)
2-5 µL	344 (35.9%)	583 (60.9%)	31 (3.2%)
X^2 statistic, p-value	*93.299, **p < 0.00001***		

Conclusions: Overall, samples with volumes < 2µl resulted in lower, more optimal MAPD scores. Sample failure rates were significantly lower for samples with volume below 2 µl (1.7%) compared to samples with 2-5 µL volume (3.2%). For biopsy samples processed on NGS-based PGT platforms, aiming for a low total biopsy tube volume can assist with lowering failure rates. Other PGT laboratories should assess these trends to determine applicability across platform types.

Disclosures: The authors are employees of Sequence46.

Funding: None.

The Effect of Courier Delays on Preimplantation Genetic Testing (PGT) Sample Failure Rates

Rodriguez, S.,[1] Joshi, H.,[1] Nefalar, J.,[1] Robles, A.,[1] Garcia, A.,[1] Tormasi, S.,[1] Welch, C.,[1] Moradian, M.,[1] Phillips, S.[2]

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Objective: Embryo biopsy samples require stable conditions for sample integrity. Accordingly, samples sent to PGT laboratories are typically mailed with next day shipping services. However, shipping couriers can experience delays for various reasons. Our aim was to investigate if and how shipment delays impact the quality of PGT biopsy samples.

Design: Retrospective analysis of trophectoderm biopsy sample shipments and PGT failure rates.

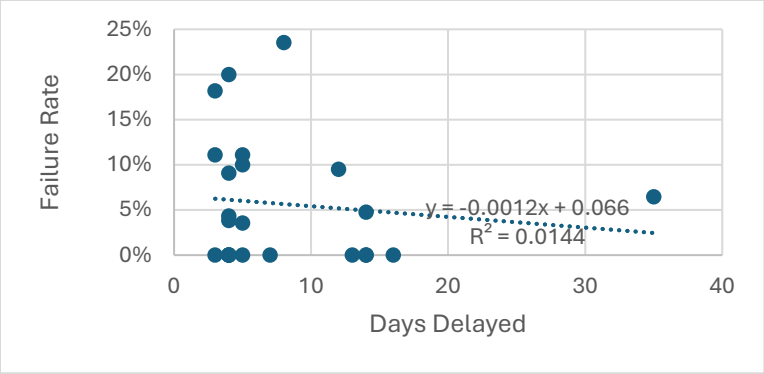
Materials and Methods: All shipments sent between November 2021 and February 2025 were included. Whole genome amplification, next generation sequencing, and data analysis were performed using the Ion ReproSeq™ PGS Kit and Ion Reporter™ software (Thermo Fisher Scientific). Shipments arriving ≥3 days after the scheduled delivery date were considered delayed. Dry run, returned, and lost shipments were excluded. Weather conditions were categorized into Cooler (Oct–Mar) and Warmer (Apr–Sep) months. Data was tallied and chi square analyses were performed.

Results: A total of 2,998 shipments were included. Table 1 and Figure 1 summarize the relevant data.

Table 1: Summary of Delayed Shipment & Failed Sample Data

Delayed Shipments (≥3 days)	30 / 2,998 (1.03%)	
Delay Length (days)	Mean: 7.81 Median: 5 Range: 3 – 35	
Failed Sample Rates	Delayed: 26 / 469 (5.5%) Range (per shipment): 0% - 23.5%	X^2 statistic = 30.322 *p-value < 0.00001*
	Non-Delayed: 1,130 / 57,355 (1.97%)	
Delayed Shipments by Climate	Cooler Climate: 19 / 1,556 (1.2%)	X^2 statistic = 1.106 p-value = 0.293 (NS)
	Warmer Climate: 12 / 1,442 (0.8%)	
Failed Delayed Samples by Climate	Cooler Climate: 12 / 305 (3.9%)	X^2 statistic = 4.314 *p-value = 0.038*
	Warmer Climate: 14 / 164 (8.5%)	

Figure 1: Failure Rate vs. Days Delayed



Conclusions: Overall, the likelihood of shipment delay using standard couriers is low (~1%). Though sample failure rates were higher for delayed shipments, most samples (~95%) still yielded successful results, with no correlation between delay length and failure rate. Samples delayed during warmer months had higher failure rates, indicating that increased ambient temperatures may contribute to greater sample degradation.

Disclosures: The authors are employees of Sequence46.

Funding: None.

The Effects of Different Sperm Preparation Techniques on Reactive Oxygen Species and DNA Fragmentation

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Objective: This is a prospective study aimed at assessing the effect of different spermatozoa preparation techniques on semen sample concentration, morphology and motility, sperm DNA fragmentation (SDF), and the presence of reactive oxygen species (ROS).

Design: Prospective study in a private assisted reproductive technology program.

Materials and methods: Semen samples were collected from 20 patients at Prisma Health – Upstate Fertility Centers of the Carolinas. After allowing liquefaction (20 to 30 min), initial concentration and motility were evaluated. Minimum sperm motility of 50% and a concentration above $15x10^6$ sperm/mL were considered for patient inclusion in the trial.

Each semen sample was diluted up to 5.5 ml sperm wash medium (Origio) and baseline concentration, motility, and morphology were determined. The sample was then split into treatment aliquots and evaluated by two traditional techniques, direct swim-out, and gradient/swim-up, and three devices: ZyMōt® (ZyMōt Fertility Inc.), Lenshooke® CA0 (Lenshooke), and NovoSort (Reproductive Solutions). Semen characteristics were assessed after each treatment as follows: sperm concentration and motility were assessed using a sperm class analyzer (SCA®, Microptic S.L.); spermatozoa morphology (Wright-Giemsa stain at 400X); ROS using the nitro blue tetrazolium assay (Oxisperm®, Halotech®); and SDF by a sperm chromatin dispersion assay (Halosperm®, Halotech®) were assessed independently by two technicians.

Datapoints were normalized as a ratio of the baseline. Data were analyzed as a mixed model with a main effect of treatment, blocked by patient. Significance was set at $p<0.05$.

Results: All endpoints showed significant treatment effect, indicating differences between the techniques for each variable. However, there was not one technique that outperformed the others in all endpoints. Swim out yielded the highest concentration compared to the other techniques, but also showed significantly lower motility and morphology and higher ROS and SDF ($p<0.05$).

Conclusions: These results provide information that could be used to select the best technique to apply depending on the intended purpose of the semen sample. Further research is needed to determine the repeatability of these results in different laboratory settings.

Disclosures: Nothing to disclose

Funding: Prisma Health – Upstate OB/GYN Department Grant, Clemson University Creative Inquiry Program

Handling Non-Expanded Blastocysts: A Comparison of Immediate Biopsy and Extended Culture Approaches

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Objective: To determine the beneficial strategy of handling non-expanded blastocysts intended for biopsy.

Design: Retrospective study set in a single academic center.

Materials and Methods: The study includes 122 cycles from 2022-2024 in which genetic testing of embryos was planned. Embryos were obtained via ICSI or conventional IVF, cultured in a single-step medium, and monitored using time-lapse system. Zona breaching was performed on day 4 to facilitate embryo hatching.
On day 5, non-expanded blastocysts were randomly assigned to immediate biopsy (Group 1, n=96) or extended culture until day 6 (Group 2, n=102), based on embryologist's discretion. Embryos were eligible for study if, at the time of decision making, up to 5 trophectoderm cells had been extruded from the zona. Genetic testing outcomes, survival and implantation rates of transferred euploid embryos were analyzed to assess diagnostic success and potential embryo trauma associated with the selected approach.

Results: All 198 embryos included in the study were successfully tested, with no samples yielding inconclusive results. Euploidy and mosaicism rates were comparable between the two approaches, at 39.6% and 10.4% in Group 1 and 36.3% and 10.4% in Group 2, respectively ($p>0.05$). Although aneuploidy rates were similar, abnormality distribution differed: complex abnormalities (≥3 chromosomes involved) were more frequent in Group 1 (33.3% vs. 13.6%, $p=0.05$), whereas isolated mono/trisomies were predominant in Group 2 (29.2% vs. 40.9%, $p=0.34$). Notably, after an additional day of culture, 13.7% of embryos left to progress degenerated, while 25% of blastocysts completely hatched out of zona pellucida, raising questions about the benefits of the "leave to grow" approach.
Twelve embryos from Group 1 and eight embryos from Group 2 were thawed for transfer. All survived, providing typical for euploid embryo implantation rates (66.7% in Group 1 and 75% in Group 2).

Conclusions: Biopsy of non-expanded blastocysts, when performed by a skilled operator, is a safe and effective alternative to extended to day 6 culture. This approach yields comparable no-call, euploidy, and mosaicism rates, does not compromise embryo survival or implantation, and may be a method of choice for high-volume IVF laboratories equipped with time-lapse systems.

Disclosures: Nothing to disclose

Funding: None

Improved FET outcomes for biopsied blastocyst transfer using EmbryoGlue instead of buffered transfer medium; direct exposure of embryonic cells to hyaluronan (HA) may be beneficial

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Objective: Comparing pregnancy rate with the use of EG verses buffered media in the transfer of biopsied blastocysts.

Design: Retrospective study in private IVF program.

Materials and methods: IVF PGT with ICSI, assisted hatching (AH), continuous culture. Principal culture media: GlobalTotal (CooperSurgical) and Complete CSC-NX (FujiFilm). Warming of biopsied blastocysts occurred approximately 3-4 hours before transfer. Ultrasound guided transfer via a 23cm Wallace SurePro catheter/introducer set (CooperSurgical). Warmed embryos were cultured using primary culture media under oil. 500ul glass syringes and transfer catheters were rinsed with buffered medium (HEPES or HEPES/MOPS). Immediately before transfer, blastocysts were washed into 1.5 ml buffered medium (HEPES, HEPES/MOPS; CooperSurgical, FujiFilm) or EG (retrospective, not randomized). Blastocysts were loaded in approximately 30ul of transfer medium, air bubbles distal and proximal to embryos. Exposure duration of embryos to buffered medium or EG, from time of washing into a well dish, catheter loading, and uterine placement was approximately 5 minutes.

Results: Outcome metrics (all ages) were calculated for biopsied blastocyst FET cycles 2012 to date, stratified only per use of buffered medium or EG as the transfer medium (TABLE). Data were de-identified and collated retrospectively. Comparisons between clinical metrics were performed using Fisher Exact Test, significance at $p<0.01$.

Clinical metrics stratified by transfer medium; buffered or EG

	HEPES, HEPES/MOPS	***EmbryoGlue***	
# Transfers	**557**	**402**	**Fisher Exact Test**
# Chemical +	**372/557 (66.8%)**	**326/402 (81.1%)**	**p<0.01**
# Clinical +	**274/557 (49.2%)**	**264/402 (66.1%)**	**p<0.01**
# Ongoing +	**236/557 (42.4%)**	**232/402 (57.4%)**	**p<0.01**
Mean embryos/FET	**712/557 (1.3/ET)**	**439/402 (1.1/ET)**	
Clin implant/embryo	**323/712 (45.4%)**	**261/439 (59.5%)**	**p<0.01**

Differences across rows, significant at $p<0.01$

Conclusions: In this laboratory, a significant positive association was observed when EG was used as the transfer medium instead of buffered media. Warmed biopsied blastocysts have breached zone, and the embryonic cells are exposed directly to culture and transfer media. Presumably large molecular weight hyaluronan in EG cannot cross intact zonae; therefore, we question if direct, immediate contact between embryonic cells to HA at the time of transfer, even briefly, may be beneficial by 1) immediate receptor mediation, and/or 2) chemical and/or physical mechanisms. Additional data reviews for all transfer cycles are planned, with attention paid specifically to condition of zona pellucidae; notations of artificial or natural breaching of the zona at time of transfer may help clarify these study observations.

Disclosures: Nothing to disclose.

Funding: None.

Ovarian Tissue Cryopreservation (OTC) for Fertility Preservation

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Objective: Vitrification of ovarian tissue cortex for fertility preservation.

Design: A longitudinal study.

Materials and Methods: Ovaries from ovariectomy received locally or from remote locations were utilized. MHM-C (Fujifilm), ES, and VS media are used for the vitrification process. ES contains ethylene glycol, DMSO at 7.5%, and VS at 20% respectfully. MOPS is also used with HSA at 10%. The Kitazato ova cryo device was used as the tissue carrier in an open system. The ovary must be dissected carefully at room temperature using media to keep the tissue saturated. In a 100mm dish, cut along the equatorial line butterflying the ovary using a surgical blade. While holding the ovary in place with tweezers gently scrape the medulla. As needed replace the 100mm dish with a new dish filled with MHM-C. These dishes will be searched for oocytes for vitrification and IVM. The medulla should be completely removed from the cortex using surgical scissors. A 1mm thin cortex cut into 1cm x 1cm sized pieces should be obtained. Wipe the tissue pieces with gauze to remove excess media. Next, place the tissue into a 10ml tube of ES and place it on a rocker for 15'. After 15' check to make sure all pieces have sunken to the bottom of the tube. If they have not the tissue must be left to rock for an additional 5'. Repeat these steps using 10ml of VS. Wipe the tissue with gauze and place it flat onto the device maximizing its surface area. Next plunge it into LN2 and store the tissue.

Results: Since 2019 a total of 57 patients ranging from 10 months to 51 years old have had their ovarian tissue cryopreserved. 26 of these patients have had oocytes cryopreserved. The other 31 patients were either pre-pubertal or have had prior chemotherapy resulting in a lack of oocytes at processing.

Conclusion: OTC is an effective procedure to preserve fertility. Some patients have also vitrified oocytes during the tissue processing. Thawing and transplanting this cryopreserved tissue has resulted in many live births. It allows patients to conceive naturally without ART. Many of these patients are cancer patients that underwent chemotherapy or are going to have chemotherapy. Having ovarian tissue cryopreserved gives these women a chance to start a family and live a normal life as cancer survivors.

Disclosures: None

Funding: None

Phased Long-Read Sequencing for Comprehensive Genetic Profiling of Men with Infertility

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Objective: This study aimed to leverage long-read (LR) sequencing to identify single nucleotide variants (SNVs), structural variants (SVs), copy number variations (CNVs), and short tandem repeats (STRs) in infertile men, with a focus on challenging genomic regions such as the Y chromosome.

Design: We conducted a prospective analysis of 170 men with diverse infertility phenotypes, including azoospermia, oligoasthenoteratozoospermia (OAT), and asthenozoospermia. All participants underwent complete clinical and endocrine assessments prior to genomic profiling.

Materials and Methods: High molecular weight genomic DNA was extracted from whole blood and sequenced to >30× coverage using library kit (SQK-LSK114), flow cells (FLO-PRO114M), and a PromethION P24 sequencer by Oxford Nanopore Technologies (ONT). Reads were aligned to the GRCh38 reference genome with Minimap2, while the T2T-CHM13v2.0 assembly was used for enhanced Y chromosome analysis. SNVs, SVs, CNVs, and STRs were called using Clair3, Sniffles2, Spectre, and Straglr, respectively. Variants were annotated using Ensembl's Variant Effect Predictor and variant classification was performed according to the American College of Medical Genetics guidelines.

Results: LR sequencing uncovered clinically significant variants not readily detected by short-read (SR) platforms. In silico karyotyping identified aneuploidies (47,XXY; 48,XXYY) and partial Y deletions (46,X,del(Y)(q12)). Multiple Y-chromosome microdeletions and microduplications (AZFa, AZFc gr/gr, and b2/b4 rearrangements) were detected. Additionally, several pathogenic SNVs were identified in genes vital to spermatogenesis and endocrine function. Notable findings included a homozygous frameshift in the *LHB* gene associated with hypogonadism, compound heterozygous *CFTR* variants in patients with obstructive azoospermia, a functionally damaging missense variant in *GHR* linked to delayed puberty, and compound heterozygous variants in many additional male infertility-associated genes. Clinically actionable secondary findings were also made. These insights offer key avenues for personalized counseling and targeted therapeutic strategies.

Conclusions: LR sequencing markedly improves the detection of pathogenic variants underlying male infertility, especially within repetitive and structurally complex regions of the Y chromosome. LR sequencing's superior ability to haplotype alleles over conventional SR sequencing technologies provides an unprecedented and crucial view of compound heterozygous variants in recessive genes. With recent CMS/CLIA approval of this ONT-based method in our lab, its clinical adoption holds promise for more accurate diagnoses and informed treatment decisions in the field of reproductive medicine.

Disclosures: The authors declare no conflicts of interest.

Funding: Supported by NICHD R01HD106056 to T.X.G.; David Sokal Innovation Award from Male Contraceptive Initiative to T.X.G.; and BCM internal funds to T.X.G.

Culture strategy relationship with the anti-apoptotic mechanism of embryonic Bcl2 and embryo self-correction

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Objective: To compare embryo culture strategy impact on the apoptosis pathway proteins Bcl2 and Bax using the mouse as a model.

Design: Experimental analysis performed at an embryology training lab.

Materials and Methods: This study consisted of a mouse embryo analysis (MEA) with time lapse imaging (TLI) used to capture morphokinetics. Mouse one-cells were randomly allocated to one of three culture groups: group, single, or culture stress group cultured in 500nM H_2O_2. Morphokinetic and blastocyst formation rates were compared. Student's t-test was used to determine significance of TLI study findings, with $p<0.05$ considered significant.

Resulting blastocysts from each group were used for quantitative polymerase chain reaction (qPCR) or immunofluorescence (IF) assays to compare resulting mRNA and protein levels of Bcl2 and Bax.

Results: To date, 168 mouse 1-cells were cultured and assessed for morphokinetics using TLI. A total of 80 embryos were cultured in groups, 72 were cultured individually, and 16 were cultured in the culture stressor 500nM H_2O_2. Group and single embryo culture blastocyst formation rates were 73.75% and 73.61% respectively. The culture stress group resulted in a blastocyst formation rate of 50%.

A total of 95 lysates were generated from 95 individual mouse blastocysts, 48 from group, 42 from single, and five from H_2O_2 exposed cultured blastocysts, for the qPCR analysis. Of the 34 analyzed, 18 lysates resulted in Bax amplification only, along with Gapdh used as a housekeeping gene. Several attempts at repeat qPCR analyses were made to detect Bcl2 amplification in all samples, with a total of three different commercially provided assay sets for Bcl2. From these repeat analyses, 16 cultured lysates resulted in successful amplification of Bcl2 that was slightly increased in the single and H_2O_2 culture groups by 11.9% and 15.5% respectively.

For the IF analysis, nine group cultured blastocysts, seven single, and one blastocyst exposed to H_2O_2 were stained for relative protein levels of Bax and Bcl2, with NucBlue (Thermo Fisher) used to stain the nuclei. Of those stained, 16 were imaged and seven analyzed using Fiji software.

Conclusions: Single embryo culture was not an altered culture state as compared to group culture, with similar morphokinetics and a slightly increased potential toward an anti-apoptosis state.

Disclosures: Nothing to disclose

Funding: Serono CRB Andrew Runge Memorial Award Recipient

Effects of Two Different Culture Media on the Rates of Fertilization, Blastocyst Formation and Euploidy in IVF

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Objective: To evaluate the performance of two culture media: Media1 (Global® medium) and Media2 (ARTSMedia In Vitro Culture Medium (AM-IVC Medium)).

Design: Comparative analysis of sibling embryo development in two distinct culture media: A randomized prospective study in a private assisted reproductive technology program.

Materials and Methods: Sibling oocytes from 58 In Vitro Fertilization (IVF) cycles between June and November, 2024 were obtained from patients undergoing fresh IVF procedures. Oocytes were cultured in either Media1 or Media2 immediately post-retrieval. All oocytes were fertilized utilizing the Intracytoplasmic Sperm Injection (ICSI) technique and were left to grow until they reached the blastocyst stage (Days 5 – 7). The handling of oocytes per case was performed by a single embryologist and the culture was conducted in the same chamber of the benchtop incubator. Fertilization rate only included oocytes fertilized with 2PN. Similarly, blastocyst formation rate included only usable blastocysts.

Results: Fertilization, blastocyst formation, and euploidy rates were compared between cohorts using a Chi-square test to determine significance ($p<0.05$). A total of 1094 sibling oocytes from 58 IVF cycles were analyzed. The Media1 group included 559 oocytes while the Media2 group included 535 oocytes. Fertilization rates were 83.9% for Media1 and 86.7% for Media2 ($p=0.187$). Blastocyst formation rates were 67.6% in Media1 compared to 55.2% in Media2 ($p<0.001$). Lastly, euploidy rates in Media1 and Media2 cohorts were 58.4% and 62.9%, respectively ($p=0.270$).

Conclusions: Among all fertilized oocytes, blastocyst formation rate was found to be significantly higher in Media1 compared to Media2. However, rates of oocyte fertilization and euploidy did not change significantly between both media. Yet, given the long shelf-life and the comparable/reproducible performance of Media2, it could also be considered as a reliable alternative for embryo culture in IVF. Further studies investigating clinical pregnancy rates are required to validate these results.

Disclosures: The authors have no conflict of interest to disclose.

Funding: None.

Fertility Treatment Outcomes using

NovoSort processing method vs. Standard Density Gradient

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Abstract

Objective: This study aims to compare conventional sperm preparation and sperm processing using a density gradient (DG) method to determine if NovoSort (isolation device, NS) improves semen parameters (WHO) post processing and proves fertility treatment success and excellent recovery rates.

Design: This study is a prospective controlled clinical trial (RCT) with patients assigned randomly with in a four-month period. The dataset compiles fertility treatment outcomes from three centers (mention above).

Materials and methods: This study consists of men undergoing ICSI, IUI, or IVF treatment with a proven history of motile sperm in their ejaculate. Exclusion criteria (seen in reference section below). All samples follow a standard lab protocol and the guidelines of the WHO standers. A total of 141 participants in this study were conducted. Male ejaculate patients from December 2024 to March 2025 were collected onsite or offside of the clinic.

Results: Across the combined dataset, NovoSort-prepared cycles showed a slightly higher pregnancy rate than Density Gradient cycles. We pool data from all centers, NS had about ~25% success vs. DG's ~17% (reflecting essentially the IVF MD results, since other centers contributed no additional updates of successful pregnancy). *While this hints that the NS method might improve success rates, the data is limited. We also observe only IUI recovery rate due to a limited number of ICSI/IVF cases in our study. Additionally, NovoSort showed equal or superior recovery of motile sperm compared to Density Gradient in the context of IUI across all centers. Overall, for IUI preparations the NS method tends to yield a higher motile sperm recovery percentage. This can be important because more motile sperm inseminated could increase the chances of fertilization. In Boca, the average post-wash recovery for IUI was 30.8% with NovoSort. (Boca didn't use DG, so no comparison there.) This means nearly one-third of the sperm were recovered as motile after NS processing. In IVF MD, IUI recovery was 18.0% with NS vs. 17.1% with DG – virtually the same, with a slight edge to NS. Both methods recovered roughly one-sixth of the sperm in motile form. In Mid Iowa, IUI recovery had a striking difference: ~43% with NS vs. ~19.8% with DG. NovoSort recovered over twice the proportion of motile sperm compared to the density gradient method for IUI at this center. This is a large gap, suggesting that in Mid Iowa's cases, NS was much more effective at concentrating motile sperm. (It's worth noting Mid Iowa's IUI sample sizes were small, so this could be an outlier result, but it does align with the idea that NS might outperform DG)

*(*Note: Each center had a different mix of NS vs. DG usage. Boca used only NS in this dataset, IVF MD and Mid Iowa had both NS and DG. Sample sizes vary widely, which will be noted when interpreting results.)*

Conclusions: The study indicates that the NovoSort sperm preparation technique has the potential to improve sperm recovery and possibly success rates in IUI/IVF treatments. At the IVF MD center, NS outperformed the traditional density gradient in terms of pregnancy rates (25% vs 17% success) and had comparable recovery efficiency. NovoSort consistently yielded equal or higher motile sperm recovery in IUI preparations across the

board, which could be beneficial for treatments. However, in IVF/ICSI contexts the advantage of NS was less clear due to the limited sample cases. Thus, we proposal a continuation of this study to further our sample size.

Disclosures: Nothing to disclose

Funding: None

Reference:

Table 1: Exclusion criteria for male participants

IUI or IVF (Conventional Insemination)	ICSI
Male sample characteristics on the day of procedure: >15m/ml concentration >30% motility >3 Progressive motility Female patient characteristics: < 40 years	Male sample characteristics on the day of procedure: >5m/ml concentration >15% motility >2 Progressive motility Female patient characteristics: < 40 years

Mouse Embryos Cultured in Media with Glycerin and Propylene Glycol

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Objective: To determine mouse embryo development in M-16 media containing common carrier fluids in vaping compounds: 1) glycerin or 2) propylene glycol alone or 3) mixed 50:50 with or without pre-vaping at 40 watts (W)

Design: Two-cell embryos individually cultured in M-16 media alone or in various concentrations of compounds #1, #2, and #3 for up to 120 hours analyzed morphologically every 15 minutes

Materials and methods: Embryos collected at 2-cell stage from superovulated (previously published) weanling aged mice were dispensed individually into 96-well plates. M-16 media of carrier fluids of the three listed compounds were dispensed at concentrations of 0%, 0.0625%, 0.125%, 0.25, 0.5%, and 1.0%. The three compounds were either vaped at 40W and condensed or unvaped (fresh). The plate was placed into a Keyence BZ-X810 incubator/microscope for up to 120 hours at 37^0, 5% CO_2, and 100% H_2O partial pressure. Micrographs were taken of each embryo at 400x every 15 minutes during incubation.

Embryo morphology was categorized as 0=degenerated/fragmented, 1=2-cell, 2=4-cell, 3=8-cell, 4=morula, 5=early blastula, 6=normal blastula, 7=expanded blastula, 8=hatching blastula, and 9=hatched blastula. Data will be recorded for statical analysis at 12-hour intervals. A paired t-test was performed between each experimental group at each point in time.

Results: Data analysis between controls and 1-0.5% compounds, either fresh or condensed vapor, for developmental levels from 8-cell to hatching blastocyst stages gave a statistically significant difference (S.D.) of 0.001. As the experimental concentrations decreased, the S.D. between controls and experimentals decreased. Significance vanished at the lowest two levels of 0.125 and 0.0625.

Conclusions: As global birth rates decline, infertility has become a growing concern for both healthcare professionals and policymakers. Concurrently, e-cigarette use among smoking women who plan for a pregnancy has increased due to the promotion of the relative safety of e-cigarettes over tobacco cigarettes. Results clearly demonstrate that e-cigarette use can be detrimental to the developing mouse embryo in constant contact at low to moderate levels of exposure. Because the vaping chemicals persist in the tissues long after the vapor has been expelled, the continual use of e-cigarettes is likely harmful to developing embryos. Future research which exposes adult mice to various vaping products will assess effects on the pulmonary as well as other systems.

Disclosures: Nothing to disclose

Funding: Internal research grant from LUCOM

Success of Monophasic Culture System versus Sequential Culture System – a 2-year Retrospective Review

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Objective: To compare the quality blastocyst and euploidy rates resulting from embryo culture in a monophasic culture system versus that of a sequential culture system.

Design: In a retrospective review of clinical data from autologous oocyte retrieval cycles in a private IVF clinic, two embryo culture systems were compared for quality blastocyst rate and resulting euploidy rate. Patient cycles analyzed from 2023 total 163 with 3237 oocytes, and in 2024 total 164 with 3312 oocytes.

Materials and Methods: In 2023, a sequential embryo culture system was utilized (Quinn's Advantage Protein Plus). In 2024, the laboratory moved to a monophasic embryo culture system (Continuous Culture NX). Medium refreshing on Day 3 continued with the monophasic culture medium in 2024. The oil for overlay during embryo culture was unchanged. pH and embryo culture conditions were optimized for both media, per the manufacturer's recommendations. Embryos were cultured in 50 uL wells under 9 mLs of oil, with 5 embryos per well in 3 incubators. All mature oocytes were ICSI'd and embryos were cultured to the blastocyst stage.

Results: Comparing the MII maturation rates at ICSI between 2023 and 2024, there was no difference (75.7% and 75.3%, respectively, p =.717). 2023 did have a higher ICSI fertilization rate over 2024 (80.0% and 75.3%, respectively, p <.05). Interestingly, though 2024 had a lower fertilization rate, the quality blastocyst rate in 2024 (64.1%) was significantly better than in 2023 (60.9%), p=.041. The euploidy rates between 2023 and 2024 were unchanged (58.0% and 59.4%, respectively, p =.517).

Conclusions: Embryo culture in the monophasic system resulted in a statistically significant improvement in the quality blastocyst rate, year over year. There was no significant improvement in euploidy rates between the culture systems. As the monophasic culture system included medium refreshing on Day 3, there is more study needed to determine any change or improvement in results with embryo culture remaining static throughout the duration of embryo culture.

Disclosures: None

Funding: None

Timing the Plunge: Impacts of Air Exposure During Ultra-Rapid Warming on Embryo Viability

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Objective: Determine the optimal timing for moving an open vitrification device from liquid nitrogen into 37°C thaw solution during the ultra-rapid warming process.

Design: Prospective, randomized, blinded study using previously biopsied and vitrified human blastocysts donated for research purposes.

Materials and Methods: Twenty-seven previously biopsied, hatched and vitrified blastocysts were randomly distributed into four groups. Each embryo was thawed using the ultra-rapid warming protocol (immersion in 37°C thaw solution (Irvine Scientific NX) for 1 minute, rinsed through warm 20% protein-supplemented handling media for 1 minute). The independent variable was amount of time the vitrification device was exposed to air before submersion into thaw solution. Devices were rapidly removed from liquid nitrogen and either plunged as quickly as possible into thaw solution (<1 second(s)), or held in air for 2s, 3s, or 5s before submersion into thaw solution. Warmed blastocysts were cultured for 24 hours (h) in a time-lapse incubator. Embryologists were blinded when assessing embryo viability and response post-thaw.

Blastocysts included in this study met all criteria: (1) cryopreserved on day five or six post-insemination, (2) oocyte donor ≤ 42 years, (3) single biopsy performed, (4) PGT-A results: aneuploid or mosaic at a single chromosome or euploid, (5) morphological grade 3BB or better at time of biopsy, (6) vitrified individually on LifeCarrier open devices.

Results: Embryos exposed to air for ≤1s and 2s had an average re-expansion time of 2-2.5h and most had hatched fully from the zona pellucida within 24h of warming (5/7 and 4/7 respectively).

Blastocysts exposed to air for 3s or 5s rarely hatched within 24h (1/6 and 0/7 respectively). Embryo atresia shortly after warming was observed for 1/6 and 2/7 embryos in the 3s and 5s group respectively. When these embryos survived the warming process, time to re-expansion was significantly slower (7.6h and 5.8h respectively) and embryos were more likely to be morphologically downgraded post-thaw.

Conclusions: For the ultra-rapid thaw protocol, vitrification devices held in air for 2s or less correlated to significantly higher survivorship and a faster and more robust recovery. Conversely, embryos warmed after 3s or 5s in air struggled to recover and progress, and were more likely to degenerate shortly after warming.

Disclosures: Nothing to disclose

Funding: None

Clinical Application of Emedgene™: An AI-Based Phenotype-Driven Approach for Prioritizing Variant Interpretation and Reporting from Whole Genome Sequencing Data in Rare Genetic Disorders

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Objective: Among the more than 8,000 rare disorders identified to date, 80% have a genetic origin. Compared to traditional array-based or targeted sequencing approaches, comprehensive sequencing methods such as whole genome sequencing (WGS) or whole exome sequencing (WES) have been shown to improve the diagnostic yield for rare genetic disorders from 15–20% to 35–55%. The process of reviewing WGS or WES findings and prioritizing variants for reporting has remained complex until recently, as distinguishing disease-causing pathogenic or likely pathogenic variants from rare benign variants is technically challenging and labor-intensive. In this study, we conducted a proof-of-concept evaluation of the Emedgene™ platform (Illumina, San Diego, CA, USA), which prioritizes disease-causing variants using a clinical phenotype-driven AI algorithm to identify variants from whole genome sequencing data.

Design: Whole genome sequencing data from patient specimens, initially sequenced and analyzed at an external commercial clinical genetics laboratory, were re-evaluated using Emedgene™.

Materials and Methods: Trio case analysis, including WGS data analysis of the proband, and biological mother and father, was performed for 20 patients using Emedgene™ (Version 37.1.1). These cases were initially evaluated at an external commercial clinical genetics laboratory as the standard of care clinical workup and predominantly contained disease-causative variants. WGS FASTQ data files were obtained from the external testing laboratory after obtaining patient consent. Sequence alignment, variant calling, annotation, and prioritization were performed using Emedgene™. Disease-causing pathogenic or likely pathogenic single nucleotide variants, indels, and copy number variants identified in the clinical workup were assessed to determine whether they were detected and prioritized with high rankings by the Emedgene™ phenotype-driven AI algorithm.

Results: Of the 20 cases evaluated, one did not contain a reportable pathogenic or likely pathogenic variant, and another contained only variants in two autosomal recessive genes that were reported. Among the remaining 18 cases, all previously reported disease-causing coding variants from the orthogonal verification platform were ranked within the top six by the Emedgene™ AI-based prioritization algorithm. Notably, in 16 of 18 (88.8%) cases, at least one of the previously reported variants was ranked as the top priority.

Conclusions: Our findings demonstrate 100% concordance between Emedgene™ and orthogonal findings, with the platform's prioritization rankings helping to streamline the clinical case review process. We intend to conduct a comprehensive evaluation using representative cases with varying degrees of pathogenicity or benign status.

Disclosures: None

Funding: None

Enhancing Efficiency and Performance in Molecular Diagnostic Testing: Practical Experience and Examples

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Objective: Share impact of process improvements yielding enhanced molecular diagnostic testing efficiency and shortened result turnaround times that enable prompt patient care interventions.

Design: Retrospective review of multifaceted process improvements in clinical NGS testing and the resulting impact on efficiency and service levels.

Materials and Methods: This study involved a collaborative, multidisciplinary team comprising Medical Laboratory Scientists (MLS), Laboratory Directors, Medical Directors, and Bioinformatics Scientists. The efficiency of nucleic acid quantification and Next Generation Sequencing (NGS) libraries was assessed using the Qubit Flex Fluorometer, and its performance was compared with the Qubit 3.0 or Qubit 4.0 Fluorometers. Performance parameters included concentration, and time required for NGS library quantification. The aim was to reduce technical time-to-data and manual pipetting effort by at least 50%, thereby increasing molecular diagnostic (MDx) testing throughput.

The in-house Hematological Neoplasms NGS (HNNGS) test was expanded for acute leukemia patients to include both DNA and RNA-based diagnostics, enabling the detection of single nucleotide variants, insertions/deletions, and gene rearrangements. To counter increased testing volume and longer TATs, multiple improvements were implemented including revising scheduling, evaluating high-throughput technologies, and upgrading Bioinformatics software. A rapid test was developed to quickly report mutations in a targeted subset of genes facilitating faster evaluation for targeted therapies and clinical trial eligibility.

Results: Integration of the Qubit Flex within NGS workflows significantly reduced sample to data time by up to 77%, thereby increasing throughput. Enhancements to the HNNGS testing workflow, reduced bioinformatics analysis time by over 50%. Introduction of NGS library preparation reagents in 96-well plate, versus 8-strip tube formats, substantially reduced the proportion of NGS results returned outside of TAT from 80% to less than 10%. Additionally, this led to a reduction in laboratory errors and improved the MLS' engagement. Rapid genetic testing for acute leukemia patients provided faster and more accurate molecular results essential for diagnosis, prognosis, and therapeutic interventions, ultimately improving patient outcomes and reducing costs. These advancements optimized laboratory operations and enhanced overall user satisfaction by delivering timely and precise results.

Conclusions: The success of these initiatives that streamlined workflows and decreased TATs is attributed to the collaborative efforts of laboratory scientists and directors, who leveraged their combined skills to continuously improve MDx testing. This underscores a people-centric approach to process improvements and the critical role of innovation and technology in advancing molecular diagnostics.

Disclosures: Nothing to disclose

Funding: None

Homologous Recombination Deficiency (HRD) Assessment Using Illumina TSO500 Assay Panel (TSO500) and Comparison of Two Analysis Software.

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Objective: To explore the application of HRD assessment in clinical diagnosis.

Design: To compared HRD assessments by using genomic instability score (GIS) obtained by TSO500 with or without HRD probe addition and analyzed using two software: Bionano NxClinical versus Illumina DRAGEN. Reference DNA and previously tested patient samples are tested by TSO500 assay with or without HRD probe pool.

Materials and methods: Four reference samples (SeraCare) and 38 clinical specimens. GIS was assessed using NxClinical software on sequencing data with ("N+H") and without ("N-H") the HRD probe pool for evaluating these genomic scars. Illumina's DRAGEN software was also used to analyze sequencing data (D).

Concordance was assessed using these cut-off criteria to determine positive GIS scores: non-Myriad method used ≥46, Myriad method used ≥42, and in-house tests used ≥42, respectively.

Results:

1. GIS of Reference DNA samples assayed using three in-house methods (N+H, N-H and D), R^2 of linear regression greater than 0.99 with labeled value. 100% concordant.
2. In-house TSO500 test with HRD probe and NxClinical GIS analysis, R^2 of linear regression greater than 0.80 compares to previous reference lab results. Concordance = 71%.
3. Inhouse TSO500 test without HRD probe and NxClinical GIS analysis, R^2 of linear regression greater than 0.79 compares to previous reference lab results. Concordance = 74%.
4. Using NxClinical GIS analysis, In-house TSO500 test with HRD probe, R^2 of linear regression greater than 0.94 compares to without HRD probe. Concordance = 94%.
5. Using Illumina DRAGEN GIS analysis, In-house TSO500 test with HRD probe, R^2 of linear regression greater than 0.95 compares to reference laboratory results. Concordance = 95%.

Conclusions: The study shows excellent concordance between in-house testing results using the Illumina TSO500+HRD kit analyzed on the DRAGEN pipeline and the reference laboratory GIS results. While concordance is slightly lower with NxClinical, the difference is not statistically significant in this small sample set.

GIS obtained using the NxClinical pipeline on sequencing data, both with and without HRD probes, shows a high correlation among samples.

The findings indicate that sequencing data from the standard TSO500 panel without the additional HRD probe pool, analyzed on NxClinical software, can be a cost-efficient initial screening method. Cases with a GIS range of 25 to 55 can subsequently be examined using supplemental HRD probes and analyzed using Illumina's DRAGEN pipeline for further evaluation.

Disclosures: None

Funding: None

Identification of an Unknown Infection Using Next Generation Sequencing Technology

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Objective: A patient with respiratory symptoms was tested negative for COVID (SARS-CoV-2), Flu (influenza viruses), and Respiratory Syncytial Virus (RSV) Infection. Both nasopharyngeal swab and sputum aspiration samples were sent to Alaska State Virology Laboratory (ASVL) for further investigation.

Design: A next generation sequencing method with Illumina MiSeq platform was used to detect potential pathogens in both clinical specimens.

Materials & Methods: A laboratory developed test (LDT) based on a patented technology (US Patent No. 11035000) was developed for investigation purpose of unknown infection. In this method, total RNAs were used for whole genomic RNA sequencing. The rational for using RNA instead of DNA sequencing is that all active infections result in RNA transcription. By sequencing RNA only, therefore, can avoid sequencing those pathogens which are not responsible for the current, active infection. Total RNAs were further enriched using this patented technology called "Preferential Amplification of Pathogenic Sequences (PATHseq®)", which can be used to greatly enrich pathogenic sequences in NGS libraries.

Results: Two pathogens, human simplex virus 1 (HSV-1) and SARS-CoV-2, were identified in the samples. Both results were confirmed at ASVL using their routine tests. The presence of HSV-1 was confirmed by PCR using Luminex ARIES system. The presence of SARS-CoV-2 was also confirmed by PCR using two separate assays: the DiaCarta QuantiVirus SARS-CoV-2 Multiplex Test and ePlex RP2 Respiratory Pathogens Assay.

Conclusions: In the future, PATHseq® technology has the potential to be used in personalized diagnosis of infection and abnormality including early cancer detection, i.e. once an individual transcriptome is established during normal (healthy) state, PATHseq® technology can be used to develop a set of non-human primers reflecting this individual health state so this set of primers can be used to eliminate normal transcripts, resulting in enrichment of pathogenic or abnormal or cancerous transcripts.

Disclosures: Nothing to disclose

Funding: None