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Abstracts

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Microfluidic Sorting Selects Sperm for Clinical Use With Reduced DNA Damage Compared to Density Gradient Centrifugation in Split Semen Samples

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Capsule: Microfluidic sorting of unprocessed semen allows for the selection of clinically usable sperm with lower DNA fragmentation than standard processing.

Objective: To determine if microfluidic sorting improves the selection of sperm with lower DNA fragmentation over standard density-gradient centrifugation.

Design: Blinded, controlled laboratory study.

Setting: Academic medical center.

Specimens: Consecutively collected routine semen analysis samples (n=70).

Intervention(s): For each sample, the unprocessed semen was tested for DNA fragmentation and split for processing by density-gradient centrifugation and sorting by a microfluidic chip. DNA fragmentation was assessed in unprocessed and processed samples by Sperm Chromatin Dispersion (SCD) test.

Outcome Measure: DNA Fragmentation Index (DFI) was calculated as number of cells with fragmented DNA divided by the number of cells counted per slide.

Results: The median DFI in unprocessed samples was 21% (IQR 14-30). In paired analyses of all samples, those processed by the microfluidic chip demonstrated significantly decreased DFI compared to those processed by density-gradient centrifugation and unprocessed samples. The median DFI for chip specimens was 0% (IQR 0-2.4) while those processed by density gradient centrifugation had a median DFI of 6% (IQR 2-11). Unprocessed samples in the highest DFI quartile (DFI range 31-40%) had median DFI of 15% (IQR 11-19%) after density-gradient centrifugation and DFI 0% (IQR 0-1.9%) after processing with the microfluidic chip (p=0.02).

Conclusion: Microfluidic sorting of unprocessed semen allows for the selection of clinically usable, highly motile sperm with nearly undetectable levels of DNA fragmentation. Future studies will be expending the sample size, and validate if the use of chip can be translated into difference in pregnancy rates.

Conflict of Interest Disclosures:

Utkan Demirci, Ph.D. is the Co-founder and Scientific Advisor for DxNow Inc., LevitasBio Inc., and Koek Biotech. Mitchell Rosen, M.D. is a member of the Clinical Advisory Board for DxNow Inc.

Funding: None.

Oocyte Cryopreservation: microSecure Vitrification (µS-VTF) No Worse Than the Rest, But is it an Experimental Procedure?

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Objective: When vitrified human oocytes yield viable blastocysts, live birth success is comparable to fresh oocytes. However, despite ASRM classifying oocyte cryopreservation as "non-experimental" in 2012, little progress has been made to understand developmental inconsistencies associated with some egg batches. Our aim was to contrast developmental incompetence of oocytes vitrified by µS-VTF in a DMSO-free solution compared to other conventional open VTF/EG-DMSO systems.

Design: Based on our limited clinical experience, we performed a retrospective analysis of our 2014 – 2018 oocyte warming-ET cycles. VTF oocytes (n=416) from 28 patients were grouped according to VTF system: open device(n=87; outside Lab source) or closed µS VTF devices (n=329; Lab control), vitrified in EG/DMSO or EG only (Innovative Cryo Enterprises, NJ) cryoprotective solutions, respectively. Differences in fertilization, cleavage, blastocyst and pregnancy rates were statistically compared between groups. In particular, we aimed to assess differences in developmental issues.

Materials and Methods: All human oocytes were vitrified and warmed using standardized, published protocols. All oocytes were ICSI'ed 2-3h post-warming and cultured in Life Global medium + protein supplementation under tri-gas, humidified incubation (37ºC) conditions up to Day 7. Blastocysts underwent ET, biopsy/PGS and/or µS-VTF/VFET.

Results: Developmental delay and reduced mean blastocyst production is common in vitrified-warmed oocytes compared to fresh oocytes (50-65%), independent of VTF method. Yet, when blastocysts are produced normal pregnancy outcomes occurred. Development incompetence between batches however, independent device-solution treatments, continues to be a serious problem (see Table results).

VTF device by Solution	Open Device DMSO/EG	µS-VTF EG+Ficoll
# Patients / # PGS cycles	7 / 4	21 / 17
# VTF oocytes	87	329
Survival Rate: #ICSI'ed (%)	61 (70%)	289 (88%)*
Fertilization Rate: #2PN (%)	43 (70%)	201 (70%)
Cleavage Rate-Day 3: #>3-cell (%)	41 (95%)	189 (94%)
Blastocyst Rate-Day 5-7: # (%)	18 (42%)	62 (31%)
Ongoing Pregnancy/LB Rate: # (%)	1/2 (50%)	7/11 (64%)
Patients without blastocysts: : # (%)	1 (14%)	4 (19%)

*Indicates significant difference between row values, X^2 ($p<0.05$); which we attribute to low #'s.

Conclusion: Delayed and compromised blastocyst development with cryopreserved oocytes continues to be a batch-to-batch problem ignored in most publications. Little experimental progress in the IVF industry is or will be made unless we initiate an ongoing scientific dialog. Ethically speaking, patients should be properly informed and consented prior to elective freeze preservation.

Disclosures: MCS developed µS-VTF without any commercial interests.

Funding: None.

Stress in the Workplace: Results from a Perceived Stress Survey of ART Laboratory Professionals

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Objective: Workplace stress is a global risk factor for workers' health and safety. Numerous studies of healthcare workers have demonstrated increased stress resulting in burnout and significant health issues. The purpose of this study was to conduct a Perceived Stress Scale (PSS) survey (Levenstein et al., 1993) of ART laboratory staff to determine the levels of stress in this group.

Design: Review of survey results.

Materials and Methods: A link containing the PSS was sent by email and posted on a social network site. Participation was voluntary. Participant responses were compiled and de-identified by the online survey tool. The PSS score was calculated for each participant with the resulting number between 0 and 1. Higher scores indicate greater levels of stress. Data was compared using unpaired t-test, with significance of $p < 0.05$.

Results: Non-physician ART lab staff (n=103) answered the survey. 96.8% were located in the U.S., 36% were Ph.D., 28% Master, 36% Bachelor degrees. Respondents identified as embryologists (n = 57), Lab Directors (n=39), and Andrology technicians (n=7). The mean score ($\pm$ SD) was .41(0.2) for Andrology Technicians, 0.51 (0.13) for Embryologists and 0.49 (0.17) for Laboratory Directors, and 0.5(0.2) for the entire group. No significant differences were seen between the groups ($p>0.05$). Embryologists were trending to higher scores than Andrology Technicians (p=0.06), IVF Lab Directors were trending to higher scores than Andrology Lab Directors (p=0.08). The lowest score was seen in the Andrology Technicians(0.12) and the highest in IVF/Andrology Lab Directors(0.93). Regional differences were also seen. Previous reports showed PSS scores of 0.36, 0.42, 0.44, 0.53 for students, healthcare workers, hospital outpatients and hospital inpatients respectively (Levenstein et al., 1993).

Conclusions: These data point to a relatively high level of stress experienced by ART lab professionals. The results and submitted comments point to a critical need to address the causes and amelioration of stress levels. Further controlled studies of a larger group are warranted. It is hoped that this preliminary report will garner increased attention to the stress level experienced by ART staff and develop means to minimize these stress levels.

Disclosures: Nothing to disclose.

Funding: None.

Utilization of Molecular Testing in Clinical Practice

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Objective: One of the goals of molecular diagnostics is integration into the clinical team to provide value added experience and personalized information for patient care. This presentation will highlight innovative ways to accomplish this goal in the filed of molecular pathology and cytogenetics.

Design:

- Overview of molecular pathology/cytogenetic testing
- Overview of molecular test ordering process in clinical and pathology practice
- Utilization and consultations service of molecular diagnostics

Materials and Methods:

- Presenting molecular diagnostic testing utilization algorithm
- Evaluating the test request
- Providing consultation for testing service, including recommendation and reflex testing if necessary
- Providing consultation/interpretation for test results
- Providing clinical assessment for patient care management, including prognostic and therapy
- Testing utilization data from UCLA, VA Medical Center Los Angeles and Kaiser Permanente
- Several case studies examples

Results: This presentation will center on molecular pathology and cytogenetic testing utilization in clinical practice. Specifically, participants will come to understand the process of evaluation test requests, providing testing recommendations and implement reflex testing in molecular pathology and cytogenetics. They will also learn how to interpret test results and make clinical assessment for patient care management, including prognosis and therapy.

Conclusions: One of the emerging trends in healthcare is precision medicine and diagnostics, whereby laboratory testing enables tailored diagnosis and treatment. Appropriate utilization of molecular testing is critical in clinical practice to provide cost-effective care and enhance patient experience.

Disclosures: Nothing to disclose.

Funding: None.

Validation of a Universal Warming/Dilution Protocol for Frozen Embryo Transfers Independent of Vitrification Device/Cryoprotectant

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Objective: Validate a universal warming/dilution approach for all vitrification (VTF) solutions associated with various device systems.

Design: Using a 2x2 factorial arrangement of treatments, 124 blastocysts derived from 363 research consented slow frozen embryos were vitrified in Glycerol/ethylene glycol (EG) solution (≥7.9M; I.C.E.) or a 15%DMSO/15%EG solution, and subsequently rapidly warmed and diluted by standard operating procedures (SOP) or a universal sucrose step-down dilution procedure (n=31 embryos/group). Furthermore, we performed a retrospective analysis of our routine success with microSecure VTF (µS-VTF with Glycerol/EG)/warming to applying a universal sucrose dilution protocol, independent of vitrification device/solution, to blastocysts shipped in from outside facilities between 2015-2017. In validating our clinical approach, we contrasted live birth rates achieved in the latter groups (A and B, respectively; see Table) to outcomes reported in the 2015 CDC data, including the national average (C) and four local IVF labs/clinics (D-H).

Materials and Methods: All research blastocysts were vitrified by µS-VTF using solutions and dilution treatments described above. Conversely, all internal FET cycles implemented a standardized sucrose step-down dilution using I.C.E. diluents (T1 -T4; estimated to possess 1.0M, 0.5M, 0.25M and 0.125M sucrose concentrations; 3 min/step), independent of the VTF device/cryoprotectant, upon rapid warming and blastocyst isolation. All blastocysts underwent isotonic equilibration in Hepes buffered media for 5 min at 37°C before being in-vitro cultured until ET or research developmental assessment. Our live birth pregnancy rates for FETs (A,B; autologous oocytes, <43 years old) were compared to both national (C) and local clinic success (D-H).

Results: No statistical differences in survival or sustained development was observed between the combined dilution treatments of vitrified research blastocysts. SOP thawing (93.5%) or standard sucrose dilution (90.3%) outcomes were similar. Mean live births (see Table) following our universal sucrose dilution approach (B) revealed similar or improved outcomes to those reported in the 2015 CDC summary (C-G). The apparent reduced live birth success compared to µS-VTF (A) and a local group (H) is likely due to differences in embryo production (i.e., lab effect), not to a warming-dilution effect post-VTF.

	A	B	C	D	E	F	G	H
# VFET cycles	384	54	36,365	87	38	424	74	164
% Live birth	71%	52%	41%	39%	45%	56%	59%	70%

Conclusion: A universal sucrose, step-down dilution approach has proven to be a simple, cost-effective and highly successful procedure in our treatment for vitrified blastocysts derived from outside IVF programs, thus alleviating the need to purchase and maintain various commercial thaw solutions.

Disclosures: MCS developed µS-VTF without any commercial interests.

Funding: None.

Variable IGF1R mRNA Expression in Individual GV and In Vitro Matured M2 Human Oocytes

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Objective: Characterization of human insulin-like growth factor 1 receptor (IGF1R) mRNA expression in individual human oocytes to better understand the role of biologically significant mRNAs in establishing oocyte quality following ovarian hyperstimulation for in vitro fertilization (IVF) treatment.

Design: Germinal vesicle oocytes (GV) were identified after removal of all cumulus cells prior to ICSI and collected from consenting patients with IRB-approval.

Materials and Methods: Single GVs were placed directly into lysis buffer (5µl). Oocytes in vitro matured (IVM) to the M2 stage were placed into individual tubes containing lysis buffer after 24 to 48 hours (h) in culture. Total RNA isolated from cumulus granulosa cells (GC) using the TRIzol reagent (Invitrogen) as stated in the manufacturer's protocol served as a positive control. Reverse Transcription (RT) was performed using anchored oligo-dT primers (IDT, Coralville, IA) and Moloney Murine Leukemia Virus reverse transcriptase (Invitrogen) on the entire 5µl oocyte lysate at 37°C for 1.5h or 1mg total GC RNA 42°C for 1h. Quantitative polymerase chain reaction (qPCR) was performed combining standards or 1µl cDNA with PCR buffer containing SYBR Green I (Sigma Chemical Co, St. Louis, Missouri), *Taq* polymerase (Genscript, Piscataway, NJ), and primers for IGF1R. Melting curves were routinely determined to ascertain generation of only the expected product.

Results: qPCR performed on GC cDNA demonstrated detection of the expected product under the reaction conditions in use. Gel electrophoresis of the PCR product confirmed the size of the DNA fragment generated. qPCR analysis detected a signal for IGF1R mRNA in 9 of the 15 GVs analyzed from 9 patients. Moreover, for 3 patients with multiple GVs, differences in IGF1R rnRNA expression were seen between individual GV oocytes, as well as between oocytes from different patients. Similarly, 8 of 12 IVM M2 oocytes from 11 patients gave a signal for the presence of IGF1R mRNA.

Conclusions: These results report the use of a direct-lysis method for the extraction and analysis of mRNA from single human oocytes. These data suggest that GV oocytes failing to respond to the hCG trigger during ovarian stimulation for IVF vary in their biological integrity. Furthermore, we observed that GV oocytes advanced to the M2 stage in vitro in the absence of any demonstrable IGF1R mRNA expression. Molecular assessment of single human oocytes can be used to better understand the low per-oocyte success rate in human IVF.

Disclosures: Nothing to disclose.

Funding: None.

Assessing the Need for Morphological Analysis of Semen Donor Specimens

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Objective: To determine the utility of sperm morphology for screening of sperm donors.

Design: Cross-sectional study in a private semen bank.

Materials and Methods: Semen samples were collected from 29 accepted sperm donors (motile sperm concentration >50 million/mL) and 29 potential donors with low motile sperm concentration (<50 million/mL). Duplicate smears from each ejaculate were stained using the Quick III™ Stain Set. All analyses were performed at 1000x by the same technician, who was blinded to the donor identities. Both strict (World Health Organization 2010) and non-strict (WHO 1992) morphology scores were obtained. The data is expressed as mean ± S.D. and was analyzed using Pearson's correlation coefficient and student's T-Test analyses, with significance set at $p = \leq 0.05$.

Results: The mean motile sperm concentration for accepted donors was significantly ($p = <0.05$) greater than rejected donors (201.7 M/mL ± 65.6 vs. 16.8 M/mL ± 10.9). The mean strict morphology score was 19.9% ± 6.7% normal for accepted donors and 10.4% ± 7.1% normal for rejected donors ($p = <0.05$). There was no significant difference in the non-strict morphology score for accepted donors (25.2% ± 8.0%) versus rejected donors (20.3% ± 30.9%) ($p = 0.209$). Abnormal strict morphology scores (≤4% normal forms) were found in 5/29 rejected donors (17.2%), but only 1/29 accepted donors (3.4%). There was a moderate positive correlation between motile sperm concentration and both strict ($r= 0.45$) and non-strict ($r = 0.51$) morphology scores for rejected donors, and a weak negative correlation between motile sperm concentration and both strict ($r = -0.09$) and non-strict ($r = -0.17$) morphology scores for accepted donors.

Conclusions: Abnormal morphology scores were uncommon, especially within the accepted donor group. For the single accepted donor with an abnormal morphology, the score was 3% normal forms, which is within the 3-4% reference interval (WHO 2010). As such, all accepted donors were considered to have normal morphology scores. The two borderline morphology scores of 3% in the rejected donor group reduces the classification to 3/29 or 10.3% of rejected donors with abnormal morphology. This study suggests that there is limited value in performing morphology analyses on sperm donors, especially those with high sperm concentrations. While assessment of morphology for sperm donor screening may be of limited utility, morphology assessment should remain a part of the clinical semen analysis.

Disclosures: Nothing to disclose.

Funding: None.

Implementation of Only Blastocyst Stage Embryo Transfers Among All Age Groups of Patients Undergoing *In Vitro* Fertilization (IVF) Treatment Cycles: An Approach to Improve IVF Outcome

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Objective: To evaluate IVF treatment cycle outcomes before and after implementing only blastocyst stage embryo transfers among patients of all age groups.

Design: IRB approved retrospective study at an academic hospital based IVF clinic.

Materials and Methods: After controlled ovarian hyper-stimulation and fertilization in vitro, zygotes produced were cultured under standard embryo culture conditions. Under ultrasound guidance, Day 3 or Day 5 embryos were transferred in appropriately prepared uterus. Our study included 2874 patients of all age groups from two different time periods (P1= 1/1/2007-9/5/2014; P2= 9/6/2014-12/31/2017). During P2, all patients regardless of their age, diagnosis, and number of embryo(s) in culture were offered embryo transfer only at blastocyst stage. Also, patients having only morulae and/or early blastocysts did not have embryo transfer during P2. These slow developing embryos were cultured to D6 and were cryopreserved if developed to more advanced stage. Patients using donor gametes, testicular/epididymal sperm, or undergoing preimplantation genetic testing (PGT) were excluded from this study. Means and standard deviations were computed for continuous measures and frequency distributions for categorical variables. For univariable comparisons of study groups (day-3 transfers vs. day-5 transfers), Pearson's chi-squared test was used to compare study groups on categorical outcomes. T-tests were used to compare study groups on continuous outcomes.

Results:

	P-1	P-2	P-Value
Number of Retrievals	2121	753	-
Number of Transfers D3 (%) D5 (%)	2016 64.7 35.3	508 4.1 95.9	-
Mean (±SD) patient age	35.0±4.7	35.3±4.2	0.18
Mean (±SD) embryos transferred	2.1±1.1	1.3±0.5	<0.001
eSET (%)	28.1	58.3	<0.001
Implantation rate (heartbeats/embryo transferred)	30.1	44.0	<0.001
Ongoing pregnancy	42.1	45.9	0.127
Ongoing multiple gestations (%)	14.2	4.2	<0.001
No transfer due to slow development	Not tracked	17.7%	-

Conclusions: 1) Transfer of only blastocyst stage embryo(s) in patients of all ages may result in high ongoing pregnancy rate even with fewer number of embryos transferred. 2) Ongoing multiple gestation rate may be reduced among patients of all age groups by transferring only blastocyst stage embryo(s). 3) Transfer of only blastocyst stage embryos allowed us to select embryos with better implantation potential, perform more eSETs and not transfer embryos evaluated with less prognostic value. 4) Transfer of only blastocyst stage embryo(s) may be a less invasive yet effective strategy to improve IVF outcomes.

Disclosures: Nothing to disclose.

Funding: None.

Increased Elasticity of the Zona Pellucida Affects Micromanipulation Procedures for Preimplantation Genetic Screening at the Cleavage Stage: Possible Role of Blastomere Alterations on Inconclusive or "No Results" Interpretations

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Objective: Occasionally, submission of blastomere(s) or trophectoderm cells for preimplantation genetic screening (PGS) may be interpreted as inconclusive ("no results"). The main reasons for those results include failure of DNA amplification, degraded DNA, or failure to load cell(s) into the biopsy tube. In a particular case at our clinic, blastomere biopsies were submitted for PGS and all embryos were labeled as "no results". Interestingly, the zona pellucida (ZP) of all embryos was extremely elastic and blastomere displacement was difficult. We theorize that the high elasticity and extended micromanipulation procedures may have affected the DNA in such a way as to interfere with the accurate assessment/interpretation of results.

Design: Case Report.

Materials and Methods: A total of 43 oocytes were harvested at retrieval. Oocytes were inseminated via standard IVF or ICSI. Ten cleavage stage embryos with a minimum of 6 blastomeres were selected for biopsy/PGS screening. Assisted hatching (AH) via partial zona dissection (PZD; tridimensional) was performed prior to blastomere biopsy. Embryo biopsy was performed via blastomere displacement.

Results: The ZP of all biopsied embryos was highly elastic. In addition, the inner layer of the ZP appeared to be detached in certain areas for some embryos. Those embryos acquired an oval-elliptical shape. The lack of rigidity prevented generation of internal pressure inside the embryo, which is necessary to displace the blastomere(s). The ZP of each embryo had to be probed at different angles in order to displace the selected blastomere(s). The time required to harvest blastomeres was extended to approximately $\geq$5 min per embryo. Selected blastomeres were eventually isolated without evidence of damage to the remaining blastomeres. All embryos were labeled as "no results" after assessment by the genetic laboratory. The report and additional options were discussed with the patients, and it was decided to proceed with embryo transfer (D5) based on blastocyst morphology. Two high quality blastocysts were transferred, resulting in a singleton term pregnancy. The remaining viable embryos were considered for vitrification.

Conclusions: Zona pellucida elasticity and extended manipulation of embryos/blastomeres may result in a form DNA degradation that interferes with the accuracy or interpretation of results during genetic assessment. Further analysis of similar cases may shed light into the relationship between difficult biopsies and inconclusive PGS results.

Disclosures: Nothing to disclose.

Intra-Technician Comparison of Sperm Morphology Evaluation

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Objective: To identify intra-technician subjective variability in sperm morphology assessment at a single site.

Design: Intra-technician evaluation of sperm morphology results compared to reported outcome on initial semen analysis using Kruger (Tygerburg) Strict criteria.

Materials and Methods: This study was a retrospective analysis of 35 semen sample slides stained using a Romanowski stain (Diff-Quick). Slides were randomly selected for semen samples analyzed January 1, 2018 – February 28, 2018 and numbered (1-35). Morphology assessment was performed by 3 andrologists, blinded to initial reported morphology results. The median percentage of normal forms (normal, $\geq$ 4%) were compared between 3 technicians to the initial reported values. Statistical analysis was performed using One-Way Analysis of Variance (ANOVA) and ANOVA on Ranks using Kruskan-Wallis test, with $p<0.05$ considered significant.

Results: Median percentage of normal forms was 4% for the initial semen analysis report. Whereas, technician 1: 3%, technician 2: 2% and technician 3: 3%. The median values among the technicians were significant ($P=0.021$). There were no differences between reported values compared to each technician. However, the median results for technician 3 and technician 2 were different ($P<0.05$).

Conclusions: The findings suggest that there is variability in assessment of sperm morphology between technicians. Inaccurate assessment reporting high percentage of abnormal forms for sperm morphology can result in misrepresented sperm data by clinicians. As a result, since low morphology is an indication for intra-cytoplasmic sperm injection (ICSI) during *in-vitro* fertilization (IVF) procedures, ICSI can be unnecessarily ordered. The additional financial cost associated with an ICSI procedure may result in financial and emotional burden for the patient. Furthermore, an incorrect abnormal morphology result leads to emotional strain on the male patient leading to the feelings of inadequacy. An increased frequency of quality assurance training of andrology technicians in groups reviewing Kruger (Tygerburg) Strict Criteria is recommended to limit assessment variability. Randomly selected images of sperm morphology from the Kruger Strict Atlas can be used to assess technician sperm morphology evaluations.

Disclosures: Nothing to disclose.

Funding: None.

Optimal Sampling Source and Time for Wet Mount Detection of *Trichomonas vaginalis* in Cervical Mucus During ART Cycle Evaluation

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Objective: Trichomonas vaginalis (TV) is a sexually transmitted parasitic disease with a global presence. Over a period of two years, 44 couples were assessed for cervical mucous (CM) quality/function as part of their infertility evaluation. Thirteen of 44 couples had TV present in periovulatory CM examination. In one case, TV was seen in fluid aspirated from the uterine cavity. The aim of the study was to evaluate the incidence of TV detection in asymptomatic cases during infertility workup.

Design: Retrospective study. All patients studied were Caucasian with the age range 29 to 39 years. Length of failure to conceive ranged from 1-9 years.

Materials and Methods: Cervical mucus specimens were aspirated from the external cervical os during the patients' periovulatory period. Wet mounts were prepared for microscopic analysis. Uterine fluid specimens were collected if indicated during sonography.

Results: Trichomonas vaginalis trophozoites were always detected in association with white blood cells (WBC) clusters or WBC's/epithelial cell clusters. In some cases, it would take several minutes to find trophozoites. Furthermore, trophozoites were similar in appearance as compared to WBC's and could only be detected by the rhythmic movement of their flagella.

Trichomonas vaginalis was identified in 14 of the women studied, 6 during IUI treatment, 4 as part of a post coital examination, 3 as part of a midcycle mucous examination and in 1 patient following aspiration of fluid in the fundus of the uterus.

All couples testing positive were treated with 2 grams of metronidazole as a one dose treatment. This treatment rendered 13 of 14 couples TV free when CM was evaluated at ovulation in a subsequent cycle. The patient not responding to a single 2 gram dose received 500 mg of metronidazole twice daily for 7 days and subsequently tested negative for TV.

Five of the 14 couples have conceived after the discovery of TV. Four patients conceived following metronidazole and one conceived in the ovulatory cycle when TV was detected. Two pregnancies have delivered healthy infants. One pregnancy is in the third trimester and one is in the first trimester. There was one first trimester spontaneous miscarriage of a tetraploid conceptus.

Conclusions: It is concluded that TV could be present with higher frequency in patients seeking reproductive treatment. It appears that antibiotic treatment enhances the chance for conception in couples so affected.

Disclosures: Nothing to disclose.

Optimizing Cryopreservation of Human Testicular Tissues

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Objective: Chemotherapy and radiation treatments for cancer or other conditions can cause permanent infertility. While adolescent and adult men have the option to cryopreserve sperm prior to treatment, this is not an option for prepubertal boys who are not yet producing sperm. Several centers in the US and abroad are preserving testicular biopsies for prepubertal male patients with anticipation that spermatogonial stem cells (SSCs) in the tissue can be used to achieve fertility in the future. In order to maximize the use of the tissue in the future, we compared cryopreservation of cell suspension to intact piece of tissue to discover the optimal cryopreservation methods.

Design: Laboratory study using human tissue.

Materials and Methods: Human testicular tissues were cryopreserved either as an intact piece of tissue by slow freezing, or as a cell suspension. The efficiency of each technique was analyzed by immunocytochemistry (ICC) for spermatogonia marker UTF1 and human-to-nude mouse xenotransplantation.

Results: The average UTF1 positive cells per gram of tissue was highest in the in fresh tissue (15.52 ± 2.5 UTF1 positive cells/gram of tissue) and it was significantly higher than any other group ($p<0.005$). From the cryopreserved groups, large tissue pieces and small tissue pieces had similar number of UTF1 positive cells per gram of tissue (9.78 ± 1.8 and 11.36 ± 4.5, respectively). There was no statistical significance between these two groups ($p=0.8$). Cell suspension had the least UTF1 positive cells per gram of tissue (2.76 ± 1.0) and was significantly worse than cryopreserved intact tissue pieces ($p<0.001$).

Conclusions: Based on the results from ICC and human-to-nude mouse xenotransplants, slow freezing of small piece of tissue is the most efficient technique to cryopreserve human testicular tissue. These studies are important because they will maximize the use of cryopreserved undifferentiated spermatogonia for future use. Intact tissue pieces have the advantage that they can be used for tissue based or cell based approaches; whereas a cell suspension can only be used for cell culture or SSC transplantation. In case organ culture or testicular tissue grafts are a viable option to restore male fertility in the future, an optimal cryopreservation technique needs to be established.

Disclosures: None.

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Potential Laboratory Cost-Savings for a Blastocyst Preimplantation Genetic Screening (PGS) Vitrification (VTF) Program

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Objective: We aimed to critically evaluate the cost benefits of a clinically proven non-commercial, aseptic closed VTF system to other commonly used open/hybrid VTF devices, and discuss the importance of cost-savings in today's assisted reproductive technology (ART) industry.

Design: Theoretical modeling of 500 PGS/VTF-all cycles was prospectively evaluated to assess costs comparing the use of a non-commercial microSecure (µS) VTF device system to three common commercial systems: Cryolock (CL), Rapid-i (R-i) and High Security Straws (HSV) VTF devices. In the analysis, we assumed a mean of 5 blastocysts biopsied per cycle yielding 2 euploid embryos for 2 vitrified ET (VFET) cycles. Media and solution costs were excluded.

Materials and methods: Costs were calculated based on protocol and prices used within our laboratory network. The CL VTF procedure used: 5 x CLs ($15.00x5=$75.00), 2 x Stripper tips ($6.17x2=$12.34) and 2 x 4 well dishes ($2.42x2=$4.84). Conversely, the µS-VTF protocol used: 5 x CBS semen/embryo straws ($2.75x5=$13.75), flexipettes ($4.00x6=$24.00) and 2 x 100mm dishes ($0.25x2=$0.50). In considering the potential use of R-i or HSV devices commonly used in the industry, we simply replaced the CL model with higher device costs ($22.50x5=$112.50). Warming costs are particularly low with the µS-VTF technique as the embryo already resides in a flexipette, requiring only: 60 mm warming dishes ($0.25x2=$0.50) and 6-well dilution dishes ($1.40x2=$2.80). Cryolock warming costs required: Stripper tips ($6.17x8=$49.36), organ well dishes ($1.70x2=$3.40), and 4-well dishes ($2.42x2=$4.84).

Results: The application of the µS-VTF offers significant cost-savings compared to our commercial use of CL devices (see Table). Based on our theoretical model (500 cycles), a total savings of up to $64,275 can be achieved depending on the commercial VTF device used.

Costs ($) / VTF Devices:	**µS**	**CL**	**R-i or HSV**
VTF / cycle	38.25[a]	92.18[b]	112.50
Warming / 2 VFET	3.30[a]	57.60[b]	59.00
Subtotal / cycle	41.55[a]	149.78[b]	170.10
Total / 500 cycles	20,775[a]	74,890[b]	85,050
Cost Savings(-) or Increase(+) ($)	- 54,115	0	+ 10,160

a, b – column values within rows with different superscripts are different ($p<0.05$; t-test).

Conclusion: Although VTF expenses represent a fraction (<10%) of a laboratory's revenue gained from blastocyst biopsy and cryopreservation, the potential savings generated using µS-VTF could support an entry level Reproductive Biologist's annual salary over 500 VFET cycles. Cost matters in today's IVF business, as long as success is not compromised.

Disclosures: MCS developed µS-VTF without commercial interests.

Funding: None.

Preparation of Semen Specimens for Cryopreservation Morning or Afternoon Specimen Collection, Washed Versus Unwashed. Which is better?

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Objective: To determine differences in semen parameters between morning or afternoon collection, response to cryopreservative addition and washed versus unwashed specimens.

Design: Retrospective analysis of sperm bank database between 2014 and 2018.

Materials and Methods: Of 1179 specimens from 22 donors, 720 were collected before noon, 458 after noon. 252 ejaculates were cryopreserved as neat, unwashed semen, while 932 specimens were gradient prepared prior to cryopreservation. A standard semen analysis was performed 30 minutes after ejaculation. Raw semen was diluted 1:1 with TEST-yolk buffer (Irvine Scientific), placed in vials, and frozen in liquid nitrogen vapor prior to storage in the liquid phase. For gradient preparation, raw semen was layered onto an 80% layer followed by centrifugation at 320g for 20 minutes. The pellet was washed, resuspended in HTF, followed by addition of the cryopreservative and frozen as above. Following a minimum of 48 hours, the test vials were thawed, and the post thaw count and % motility were determined. Data was analyzed using a 2-tailed, unpaired t-test with significance set at $p \leq 0.05$.

Results: When all donor data was compared, there was no statistical difference between morning and afternoon semen collection for volume ($p=0.006$), count ($p=0.49$), motile count ($p=0.59$) and % motility ($p = 0.95$). When AM to PM collections were compared for each donor, no statistical difference was seen for all parameters in only two donors. Volume was significantly higher in 5 donors; count was higher in AM for 2 donors and in PM for 2 donors; % motility higher for 1 donor in AM and in PM for 1 other donor. The gradient prepared specimens showed significantly ($P= 0.0001$) higher post-thaw motility and cryosurvival rate than unwashed specimens.

Conclusions: There appears to be no difference in semen specimens collected in the morning versus in the afternoon, although there was a trend for higher semen volume in the afternoon specimens. There were differences in individuals between morning and afternoon collections which should be considered when evaluating sperm donors as well as clients, particularly for use in ART procedures. Gradient preparation prior to freezing results in better motility, motile count and cryosurvival before freezing and after thaw, suggesting that this method is more favorable for donor sperm cryopreservation.

Disclosures: Nothing to disclose.

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Preventing Attrition Among Non-Technical Laboratory Employees

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Objective: In the past five years, American Esoteric Laboratories (AEL) has experienced more than 70% attrition among non-technical employees. These individuals receive the lowest pay, have the least training, and are primarily responsible for specimen accessions and processing. As an alternative to previous recruitment strategies for this employee population, AEL proposed to recruit new employees from two academic settings with backgrounds in biology, chemistry, or clinical laboratory science majors. A total of 20 employees were recruited; these new employees were surveyed after 6 months of employment.

Design: The part time employees who were recruited from the student population at the University of Memphis or Southwest Tennessee Community College were surveyed. This survey included a series of questions directed at seven (7) University student employees and 13 community college student employees.

Results: All 20 student employees responded to the survey (100%). Seven were B.S. students working part-time, and 13 were A.S. students also working part-time. All students in the survey recommended AEL for employment with high satisfaction. Outside the survey, school administrations have all received positive feedback from these employees. The overall attrition rate has dropped to 48% with in the areas of accessioning and processing since the program was initiated, and no students have left employment.

Conclusions: These preliminary findings indicate that there is a clear opportunity to improve the attrition rate among non-technical employees by targeting the recruitment towards college students.

Quantitative PCR Assays for Molecular Diagnosis of *M. leprae* and *M. lepromatosis*

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Objective: To develop molecular diagnostic assays for leprosy (Hansen's Disease) caused by *Mycobacterium leprae* and *M. lepromatosis.*

Design: *M. leprae* was believed to be the exclusive causative agent of leprosy until another species, *M. lepromatosis,* was identified in 2008. We isolated and cultured the first strain of *M. lepromatosis* in the mouse footpad (MFP), re-sequenced the genomes, and developed a *M. lepromatosis* specific Q-PCR assay. We characterized this assay, and a previously designed *M. leprae* specific Q-PCR assay, for molecular diagnosis of leprosy according to the Clinical Laboratory Improvement Amendments (CLIA) guidelines.

Materials and Methods: *M. lepromatosis* was harvested from MFP, and host DNA was depleted by 0.1 N NaOH treatment of the infected MFP homogenate prior to DNA extraction. A genomic library was prepared using a NextEra XT kit and sequenced using a V3 kit (2X300 bp reads) on a MiSeq instrument (Illumina). All the reads were first aligned with mouse genomes, and only ~24% of the reads non-homologous to mouse genomes were aligned with a previously sequenced *M. lepromatosis* genome. A genomic sequence (~200 bp) which was at much higher coverage (>2000X) compared to the average genome coverage (~136X) was identified which we hypothesized must represent multi-copy regions in the genome, and thus the potential target for highly sensitive PCR assays. A *M. lepromatosis* specific Q-PCR assay (LPM) targeting this sequence was designed. This LPM assay, along with our earlier published RLEP assay for molecular detection of *M. leprae*, were CLIA validated.

Results: Polynomial regression analysis of standard curves (n=5) for both assays have excellent linearity (RLEP: $r^2<0.9946$; LPM: $r^2<0.9995$), and efficiency of the RLEP and LPM assays is 94.82 ± 3.27 and 93.05 ± 6.78, respectively. The Limit of Detection (LOD) of the LPM and RLEP assays is 3.05 (Ct≤ 34.184 ± 0.395) and 0.76 30 (Ct≤ 34.839 ± 0.846) bacilli in the Q-PCR reaction, respectively. The assays were tested on clinical specimens and samples from wild armadillos. Only 1.4% (3 / 206) of samples from the United States and 31% (15/47) of specimens from Mexico were positive for *M. lepromatosis. A*ll 180 patient samples from the Philippines and 96 samples from US armadillos were negative for *M. lepromatosis*.

Conclusions: We have developed the first CLIA-validated PCR diagnostic assays for leprosy and have implemented them into the point of care at NHDP.

Disclosures: None

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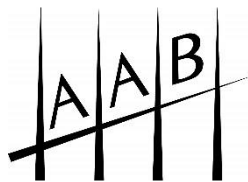

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