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Abstracts

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ABSTRACTS - ORAL

ABSTRACTS - POSTER

ABSTRACTS – POSTER, continued

ANALYSIS OF QUALITY CONTROL FACTORS AND TECHNICAL COMPLIANCE ON THE OVERALL CLINICAL EFFECTIVENESS OF MICROSECURE VITRIFICATION (µS-VTF): A 3 YEAR REVIEW OF BL-VTF/ET

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Introduction: Four years ago, the importance of Quality Control (QC) factors and the potential advantages of the **µS-VTF** system was first presented in the scientific community (Schiewe, 2009, AAB/CRB). The non-commercial **µS-VTF** method offered sterility and security using FDA compliant devices. In addition, it was safer, simpler and potentially more successful than new, over-priced commercial VTF devices. We proposed that once the **µS-VTF** system was integrated into clinical practice it would provide unprecedented intra- and inter-laboratory reliability and repeatability upon Embryologist of different experience levels.

Materials and Methods: From January 2010 – December 2012, 479 patients experienced a VTF-ET cycle in our laboratory, employing the **µS-VTF** technique by 6 different Embryologists of varying experience. Embryologists were given less than a half-day training before being expected to implement **µS-VTF** the next day. The most critical QC training steps being: 1) prefilling the VTF tip with V3 solution, expelling a small amount and then loading 1 or 2 BLs in the mid-third of the pipette volume; 2) aseptic drying of the shortened VTF tip on sterile gauze before insertion into the 0.3 mL CBS Embryo straw (label end down, creating an air space on the open end); 3) effective sealing while securing the straw from vibration; 4) inversion of straw label-end up to confirm ease of a dry VTF tip to drop freely to the sealed base-end; and 5) viewing air space between the hydrophobic plug and VTF tip for the presence of liquid droplets. Liquid on the sidewall of the inner straw could be potentially problematic in two ways: 1) could cause the VTF tip to stick to the straw which would inhibit rapid warming; and 2) droplets could have been created by accidentally sealing the pipette base with the straw, if not shortened or moved forward to the plug before sealing, thus expelling the contents, with BLs/oocytes, into the straw. The importance of QC step #5 is that if a potential issue or problem is identified, notes can be written onto the Cryo datasheet to make sure that troubleshooting measures are applied upon warming to still optimize survival. The latter check-n-balance is an advantage of using an aseptic, closed system. Finally, upon warming, the continued QC steps included: 6) easy selection and secure identification of a desired straw by reading dual-colored, internalized labeling; 7) using a LN_2 filled 0.5 L stainless steel Dewar flask (Hampton Research, Aliso Viejo, CA) to contain the patients' cane/straws, the selected straw is grasped below the label/plug with a Mayo scissor, the scissor is tapped a couple times against the dewar flask before removal; and 8) straw is cut and quickly tipped downward over a warm 0.5M sucose bath (15 mL in a 60 mm petri dish) allowing the VTF tip to free-fall into solution to facilitate rapid warming (6000°C/min). Moisture in the straw will inhibit the rapid evacuation of the VTF tip. Pre-VTF laser collapsing of BLs was not preformed prior to VTF in a glycerol-based solution (I.C.E., Linden, NJ), however laser hatching was performed before culture (1-3 hr) and ET, if needed.

Results: Each technician easily integrated **µS-VTF** into their skill sets with equal reliability. Of the 963 BLs warmed and recovered (100%), 925 survived (96%). Over all age groups (up to 43 y.o.) and a mean of 1.8 BLs transferred, we experienced a 69% CPR, 59% LBR and a 50% implantation rate. All straws were correctly identified, and nearly all VTF tips freely evacuated the straw by adhering to QC steps #2, 7 and 8. However, it is worth noting that complacency in QC step #1 (i.e., loading BLs too close to the tip), has resulted in a couple incidents where BLs were safely found in the thaw bath, not in the tip/T1 solution, without compromise.

Conclusion: In 2012, we experienced 99.2% survival of PGS-biopsied BLs, suggesting that selective collapsing of zona-intact expanded BLs may be warranted to optimize survival. The overall effectiveness of this technology was dependent on organization (i.e., clarity of mind), attention to detail (QC steps; consistency) and reliable execution (i.e., focus and concentration). The **µS-VTF** device has proven to be highly effective in several laboratories now, offering simplicity, technical repeatability, low costs and distinct QC advantages.

COMPARISON BETWEEN DAY 4 AND DAY 5 EMBRYO TRANSFER AFTER BIOPSY

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Introduction: The aim of this study is to compare the clinical pregnancy rate of day 4 versus day 5 embryo transfer after Preimplanation Genetic Diagnosis (PGD) for 5 chromosomes aneuploidy. Day 4 embryo transfer was performed to avoid working in the weekends, public holidays and subject to availability of a certain gynaecologist according to patient's request.

Materials and Methods: All of the patients who were accepted for ICSI and PGD programme were allocated to either day 4 or day 5 transfer (April 2010 to November 2011). On day 3, all of 6-8 cell stage embryos were done laser hatching by class I non contact infrared LASER (300 mW, 1480 nm) by ZILOS-tk (Hamilton Thorne USA); after that a single blastomere with a visible nucleus was biopsied from each embryo. Then the embryos were transferred to blastocyst culture medium, each embryo in a separate drop. All of the embryos were cultured in sequential media (Cook, Ireland). The culture was done in 6% CO_2, 5% O_2 and 89% N_2. The morphological assessment of day 4 embryos was done in reference to Feil et al (2008). The assessment ranges from A-F, where A is the best. All of the transferred embryos were screened by PGT kit (Vysis USA) for chromosomes 13, 18, 21, X and Y. Only normal embryos were considered for transfer. All of day 4 transferred embryos had score A-D. 1-2 embryos were transferred. The left over embryos were vitrified.

All day 5 morphological assessment was done according to Gardner and Lane (2000). The score for the transferred embryos was 5AA – 3BB.

The number of patients for day 4 transfer (group 1) was 57 with average of 1.8 embryos per transfer, and the number of patients for day 5 transfer (group 2) was 108 with average of 1.7 per transfer. The mean age of group 1 is 32.3 versus 31.9 for group 2. Student T test and Fisher's exact test was used for statistical comparison.

Results: In group 1, 519 embryos were cultured (average 9.1 / egg retrieval), 196 (38%) of them arrested at day 3. The number of embryos that reached early blastocyst stage (class A) is 21 (4%). 18% of them were complete morule and 37% had varying stage of compaction (grade C- E). The number of clinical pregnancies is 27 (49.1%).

For group 2, 961 embryos were cultured (average 8.9 / egg retrieval). 381 (40%) embryos arrested on day 3. The number of embryos that reached different stage of blastocyst on day 5 is 491 embryos (51%), the remaining 9% were at morula stage on day 5. The number of clinical pregnancies is 55 cases (51%). There was no statistical difference between the two groups.

Conclusion: Day 4 embryo transfer can be used as an alternative to day 5 embryo transfer after PGD for 5 chromosomes aneuploidy.

EFFICACY OF A SMALL, LOW-COST MICROCHAMBER FOR EMBRYO CULTURE WITHIN A NON-CO_2 ENVIRONMENT

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Introduction: In the laboratory, a stable culture environment is imperative for embryo development and in vitro success. Traditional incubators, even small desk-top models, require a constant flow of mixed gases to maintain media pH and therefore mimic an *in vivo* environment. In addition, incubator door openings may perturb the internal environment to the extent that embryo development is compromised. The efficacy of an innovative, low-cost way to culture embryos was assessed using a prototype of a sealed, pressurized device called a Microchamber (Digital Medical Instruments; Tonawanda, NY).

Methods and Materials: The pH and temperature stability was assessed over time prior to using the Microchamber to culture embryos. Temperature readings were taken once every 10 sec throughout all studies with a micro-thermocouple wireless data logger (Marathon Products, San Leandro, CA) immersed in oil. The pH of a bicarbonate-buffered media was measured daily using an i-STAT blood gas analyzer (Abbott POC, Princeton, NJ). Thawed one-cell mouse embryos (n=106; Embryotech, Haverhill, MA) were allocated into media (Global; IVF Online, Guilford, CT) with 10% protein (SSS; Irvine Scientific, Santa Ana, CA) in GPS dishes (IVF Online) under oil, and placed either into the Microchamber, which was gassed for 2 min with 6.5% CO_2/5% O_2/88.5% N_2, sealed, and placed within a heated, non-CO_2 Sanyo incubator, or into our standard culture system using small, triple gas-charged, glass, vacuum desiccators placed in a front-loading Hera-Cell CO_2 incubator. Embryo development was evaluated daily for 120 hours.

Results: Temperatures in both the microchamber and the desiccator remained constant at 36.9±0.1^{o}C throughout the culture period. pH was very similar in the Microchamber (7.26±0.01) and desiccator (7.27±0.01). The pressure in the microchamber was kept between 1.2 and 1.6 psi throughout the study. No difference in mouse embryo development was observed between the two culture systems. Blastocyst formation rate on day 4 was 88.7% (47/53) in the Microchamber and 90.6% (48/53) in the desiccator ($p=0.75$). The rate of blastocyst hatching on day 5 was 81.1% (43/53) for both the Microchamber and desiccator.

Conclusions: The Microchamber was as effective at maintaining pH and temperature stability and supporting mouse embryo development as our traditional method of culture. It is reasonably priced (projected cost <$500/device), and cost effective to operate due to the limited amount of gas required to equilibrate the small chamber (1/10 the volume of a desiccator and 1/1000 the volume of the incubator), and the ability to culture in an incubator without an additional gas feed.

Conflict of Interest Disclaimer: No author has a financial interest in any of the products used in this study.

MALE FACTOR INFERTILITY IS ASSOCIATED WITH HIGHER INCIDENCE OF NUCLEAR FRAGMENTATION AND MICRONUCLEATION BUT NOT BINUCLEATION IN CLEAVAGE-STAGE EMBRYOS

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Introduction: Multinucleated blastomeres are observed in cleavage-stage embryos on day 2 and 3 of development. A high incidence of multinucleation has been associated with abnormal embryo development, in particular fragmentation and cleavage arrest as well as high rates of chromosomal abnormalities. Multinucleation can be a consequence of nuclear fragmentation driven by apoptosis, micronucleation due to mitotic dysfunction when one or more chromosomes lag behind in anaphase, and binucleation from incomplete cell division (karyokinesis without cytokinesis).

Objective: The aim of this study was to assess whether paternal factors are related to the multinucleation rate of day 3 cleavage stage embryos using FISH analysis of cells biopsied for preimplantation genetic screening.

Design: Retrospective review of oocyte donation cases (n=137) with 37% male factor.

Materials and Methods: Single cells (n=1192) from embryos with ≥5 cells were fixed onto slides, hybridized with chromosome-specific FISH probes and counterstained with DAPI. Nuclei were analyzed directly at the microscope and checked with digital images captured with Cytovision FISH system.

Results: Frequency of nuclear fragmentation and binucleation amongst biopsied cells were similar (4.2% and 4.8% respectively) and higher than micronucleation (1.8%). Male factor (fresh or pre-freeze SA parameters: count $<20 \times 10^6$/mL, and/or motility < 40% and/or strict normal morphology <5%) was associated with an increased rate of multinucleation, both with fresh (14% vs. 8%, $P<0.05$) and frozen (15% vs. 9%, $P<0.05$) sperm. Specifically, nuclear fragmentation and micronucleation were significantly increased with male infertility (6.0% vs. 2.9% and 3.2% vs. 0.9%, respectively), whereas binucleation was not (5.0% vs. 4.4%). There was no relationship between male age (range: 29-80 y) or sperm DNA fragmentation index and incidence of multinucleation. Binucleated cells were sub-divided according to whether the 2 nuclei were: (1) both euploid, 39% (2) equivalent aneuploid, 28%; (3) different aneuploid, 30%; and (4) euploid/aneuploid, 4%. None of the sub-groups correlated with a male factor. Interestingly, group (1) were biopsied from higher quality embryos (mean of 8.1 cells and 29% grade A) compared to groups (2), (3) and (4) (combined mean of 6.5 cells and 3% grade A).

Conclusions: Male factor infertility was associated with nuclear fragmentation and micronuclei formation in day 3 blastomeres. Studies have shown that abnormal head-tail junctions and fibrous sheath dysplasia in sperm may be linked with dysfunction of the paternally inherited centrioles in the zygote. Chaotic mitotic divisions that can lead to cleavage block and cell death are common during early embryo cleavage and may arise from male-mediated centrosome, spindle or cytoskeletal dysfunction. Support for our findings comes from the micronucleus assay, which is a widely adopted genotoxic test and shown to assess paternally transmitted effects on early embryos. Binucleation was not related to male infertility, although further data is needed to elucidate the origin and significance of this heterogeneous group.

OOCYTE QUALITY AND CLINICAL PREGNANCY ARE ASSOCIATED WITH FOLLICULAR FLUID METAL CONCENTRATIONS IN IVF PATIENTS

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Background: Metals are a ubiquitous component in our environment. However, safety studies have not focused on the potential reproductive effects of metals as much as other possible target systems.

Aim: To develop a fast, multi-element screening and testing method to evaluate human follicular fluid for metal concentrations. Secondly, to determine if follicular fluid metal concentrations are associated with IVF outcome.

Method: Approximately 230 human follicular fluid samples were analyzed for 21 metals using Inductively Coupled Plasma-Mass Spectroscopy as a single multi-element method. A 0.01% EDTA sample matrix matching technique was used to calibrate for and analyze metal concentrations. Associations between the metals and demographic and embryology data were assessed. Statistical tests included logistic, linear, and mixed effect logistic regression analysis, and $p<0.00238$ was considered significant.

Results: Eighteen of the 21 metals evaluated were found in human follicular fluid. The concentrations of several metals were found to have significant effects on three of the fourteen reproductive endpoints assessed. Decreased oocyte maturity was associated with elevated levels of Vanadium (-0.053%/ppb ± 0.012, $p <0.001$), Manganese (-0.12%/ppb ± 0.019, $p <0.001$), and Arsenic (-0.062%/ppb ± 0.019, $p = 0.0011$). Nickel (-0.025%/ppb ± 0.0066, $p = 0.0002$) was negatively associated with embryo development on Day 2. Conversely, Strontium (OR 1.34, CI: 1.14-1.57, $p <0.0001$) and Barium (OR 1.53, CI: 1.19-1.95, $p = 0.001$) in the follicular fluid showed positive associations with achieving clinical pregnancy.

Conclusion: The concentration of specific metals in the follicular fluid appears to be negatively associated with oocyte maturity and embryo quality on day 2, while the presence of other metals is significantly associated with clinical pregnancy. The use of a 0.01% EDTA sample matrix proved to be an acceptable matrix matching technique for analyzing follicular fluid samples.

PREGNANCY AFTER CALCIUM IONOPHORE ACTIVATION OF THE UNFERTILIZED OOCYTES AND ANEUPLOID SCREENING USING a-CGH

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Objective: To report a successful pregnancy after transfer of embryos derived from unfertilized oocytes activated by calcium ionophore and aneuploid screening using a-CGH.

Design: Case report in a Private IVF clinic.

Materials and Method(s): A 30-year-old patient diagnosed with unexplained infertility underwent IVF with ICSI. Thirteen oocytes were collected (5 MII and 8 MI or GV). Five were at metaphase II and the rest were either metaphase I or germinal vesicle. Five metaphase II oocytes were injected with morphologically normal spermatozoa. The fertilization was checked 18 hours later. Unfertilized oocytes were activated with calcium ionophore with 10 μM calcium ionophore solution for 20 minutes at 37°C in 6% CO_2. Immature oocytes that progressed to mature underwent ICSI on the morning of day 1 and were treated with ionophore immediately after ICSI. On the morning of day 3, embryos which had at least 6 cells were biopsied and sent to the genetic lab for aneuploid screening using a-CGH. Two day 5 embryos were transferred and the ongoing pregnancy was followed.

Result(s): 2 out of 5 MII oocytes were fertilized after ICSI. 3 unfertilized oocytes were treated with calcium ionophore. 4 immature oocytes progressed to MII and were injected and activated immediately after ICSI. 1 of original fertilized oocytes cleaved. All three activated unfertilized oocytes cleaved. Three out of 4 immature oocyte injected were fertilized and cleaved. Seven embryos were tested for aneuploidy. Four embryos were diagnosed as euploid, of which 2 from activated unfertilized oocytes; 1 from original fertilized oocyte and 1 from day 1 ICSI oocytes. Two normal blastocysts resulted from activated unfertilized oocytes were chosen for transfer. Clinical pregnancy was confirmed at 7 weeks of 2 gestational sacs with fetal heartbeats. Healthy babies were born on 2012.

Conclusion (s): Artificial oocyte activation using calcium ionophore is beneficial in some cases with unfertilized oocytes. This study showed that the method of oocyte activation does not affect chromosome constitution or the normal rate of growth of pre-implantation embryos. Further studies are needed to confirm the safety of oocyte activation.

Key Words: calcium ionophore, oocyte activation, unfertilized oocytes.

SPERM HEAD MORPHOLOGY (SIZE AND SHAPE) AND LENGTH-TO-WIDTH RATIOS: AN OBJECTIVE ANALYSIS FOR CLASSIFICATION

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Introduction: Sperm morphology is a subjective assessment that has been linked with fertility potential. According to the published acceptable measurements, sperm heads should have the following dimensions: length: 4.0-4.5 microns; width: 2.5-3.5 microns (WHO 5th Edition, 2010) with a length-to-width ratio of 1.50 to 1.75. A previous study (Roudebush et al., 2006) found that sperm head length-to-width ratios closely followed the golden mean (AKA golden ratio or phi), where abnormal sperm cells fall on either side of the mean of morphological normal sperm cells. To confirm this prior work and to further delineate the importance of the length-to-width ratio, we utilized a computer assisted semen analyzer (CASA) to measure the ratio between normal and abnormal sperm (head-size and head-shape) within different semen specimens.

Objective: To determine length-to-head ratios in morphologically normal and abnormal sperm head (shape and size).

Design: Observational cohort analysis of morphologically normal and abnormal sperm head (size and shape) assessed by CASA, and calculated for phi (length-to-width ratios).

Methods: Semen samples were obtained during routine male testing. A routine semen analysis, including sperm morphology (strict criteria) was performed following WHO guidelines (2010). Head length and width were determined (CASA; Sperm Class Analyzer, Microptic, Barcelona, Spain), recorded and the length-to-width ratios (phi) calculated [phi=(L*W)/L] for each sperm (200 sperm per patient). Data were analyzed by Student's t-test.

Results: A total of 1,200 sperm cells from six different individual semen samples underwent analysis as described above. There was a significant ($P<0.001$) difference in phi between sperm head shape classified (via SCA) as morphologically normal (mean 1.667 +/- 0.060) and abnormal (mean 1.618 +/- 0.147). There was a significant ($P<0.001$) difference in phi (mean +/- SD) between sperm head size classified as morphologically normal (1.668 +/- 0.069) and abnormal (1.621 +/- 0.132).

Conclusion: Sperm head length-to-width ratios (phi) for shape and size (normal and abnormal) provide a more objective assessment of sperm head morphology and may prove to be more uniform, repeatable and reliable than standard subjective assessments. Further studies are required to demonstrate the importance of phi and the prediction of fertility.

UNEXPLAINED INFERTILITY PREDICTS POOR TROPHECTODERM SCORE

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Objective
To evaluate if *in vitro* fertilization (IVF) cycle diagnosis is predictive of embryo blastulation, inner cell mass (ICM) and/or trophectoderm (TE) scores on day 5 of embryo culture.

Materials and Methods
Women undergoing a fresh, autologous IVF and/or ICSI cycle using fresh or donor sperm at Montefiore's Institute for Reproductive Medicine and Health from 1/2007–12/2010 were included. Fertility diagnoses were tubal factor, male factor, polycystic ovarian syndrome, unexplained infertility, endometriosis, diminished ovarian reserve, uterine and other. Patients with more than one infertility diagnosis were excluded. Analysis was performed for patients whose embryos were grown out to blastocyst stage and who received a day 5 embryo transfer. Observations of blastocyst expansion (good, fair, poor), ICM (good, fair, poor) and TE (good, fair, poor) were based on Veeck et al. 2004 and standardized between embryologists. Statistical methods included Pearson's Chi Square, Fishers Exact Analysis, ANOVA and multivariate logistic regression. $P < 0.05$ was considered significant.

Results
A total of 1727 embryos from 142 patients were included in our study. All patients in this cohort received a transfer on day 5 with an overall blastulation rate of 42.8%. Blastulation expansion was not different between infertility diagnoses. Embryos from women with unexplained infertility were significantly more likely to have poor TE score vs good/fair TE score following univariate analysis ($p=0.03$). Similarly, embryos from women with unexplained infertility or endometriosis were significantly more likely to have a poor ICM score vs good/fair ICM score ($p=0.02$ and $p=0.04$, respectively). After controlling for age, maximum follicle stimulating hormone (FSH), number of oocytes retrieved and number of mature oocytes, unexplained infertility was a significant and independent negative predictor of TE score (see Table).

	Odds Ratio	95% CI	P value
Unexplained Infertility	0.66	0.47-0.94	0.02*
Age (years)	1.03	1.0-1.05	0.07
Maximum FSH (IU/L)	0.96	0.92-1.02	0.18
# Oocytes Retrieved	0.95	0.91-0.99	0.02*
# Mature Oocytes	1.06	1.00-1.11	0.03*

Conclusion
Trophectoderm score has recently been shown to predict pregnancy outcomes. We demonstrate that unexplained infertility predicts poor TE grade in blastocysts, which could impact live birth outcomes. Additional analysis will be conducted to establish whether poor TE grades are associated with live birth rates in this cohort of unexplained infertility patients.

Support
None

DEVELOPMENT OF HUMAN EMBRYOS IN THE EMBRYOSLIDE™: A RANDOMIZED, PROSPECTIVE STUDY COMPARING SEQUENTIAL AND SINGLE-STEP CULTURE SYSTEMS

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Embryo culture in the EmbryoScope™ requires culture in an EmbryoSlide™, which is a well within a well system (WOW; Vajta et al., 2008). Though each well contains up to 25 µL of media, the embryo rests in < 1 µL in the inner well, presenting the clinical laboratory with a new culture paradigm, a culture model that has not been studied extensively. Most culture media is based on one of two foundations: a single-step system typified by Global based on KSOM (Biggers et al., 2000) and a sequential system typified by Vitrolife's G-series media based on work by Gardner et al., (1996). A comparison of the original formulations illustrates that energy substrates differ considerably, particularly for glucose concentrations for post-compaction stage (day 3-6). In this study, sequential (G1 Plus/G2 Plus, Vitrolife) and single-step (Global Total, IVFOnline) media were compared using an EmbryoScope™ to obtain time-lapse images from 72 pronuclear stage frozen human embryos. EmbryoSlides™ were prepared by placing the two media alternately into each of the 12 culture wells and covered with 1.2 mL of washed Global Lite Oil. Embryo images, from seven focal planes, were obtained every 20 minutes. During the culture period, slides were briefly removed at two intervals on day 3 and day 5 for media replacement. Blastocysts were vitrified, warmed (S3 Global Fast Freeze and Fast Thaw) and total cell counts were obtained.

Embryo development was assessed per conventional standards, and parameters are listed in Table 1. All parameters assessed were not significantly different between the two media. Similarly, the timings of selected cell divisions (Table 2), and results from vitrification, warming and total cell counts (Table 3) were also not significantly different.

Table 1 – Embryo Development

	Day 2				Day 3		Day 4			Day 5	Day 6
	% with MN	<4c	4c	>4c	Cell #	Gr (0-3)	Cell #	Gr (0-3)	% Blast formation	Good Blasts	Good Blasts
Global	27.7%	30.6%	52.8%	16.6%	7.22	1.06	8.15	1.69	63.9%	36.1%	47.2%
G1/G2	36.1%	27.8%	44.4%	27.8%	7.46	1.12	9.52	1.63	41.7%	41.7%	47.2%

Table 2 – Cell Cycle Timings in Hours

	Length of time to:			Length of time as:				
	2c	>4c	Blastocoel	2c to 3c	3c to 4c	4c to 5c	3c to 5c	5c to 8c
Global	27.42	51.11	98.16	12.20	1.57	11.09	12.94	8.84
G1/G2	27.35	52.83	98.81	10.63	2.41	12.57	14.89	9.22

Table 3 – Vitrification, Survival and Cell Counts

	% of Embryos Vitrified	% of Surviving Embryos	Total Cells Counts
Global	72.2%	80.7%	149.5
G1/G2	58.3%	95.2%	126.7

Overall, embryo development was excellent with nearly 50% good blastocyst formation by day 6 in both media systems. Kinetics of development did not differ between the two media, even though the two systems provide markedly different nutrients at both the pre- and post- compaction stages, indicating that, in terms of morphological appearance and cell cycle timing, human embryos are adaptable to a wide range of nutrients. This study demonstrates that blastocyst formation and cell number using a culture well system is not affected by the limited amount of glucose present in Global media during the post-compaction phase. The EmbryoScope™ was invaluable as a research tool for obtaining objective cell cycle information between treatments while maintaining a consistent culture environment.

DO INSURANCE COMPANY GUIDELINES RESTRICTING INTRACYTOPLASMIC SPERM INJECTION (ICSI) USE AFFECT IVF TREATMENT OUTCOMES IN A MANDATED STATE?

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Objective: A principle insurance agency in Illinois, one of 15 mandated states, is addressing current inconsistences in infertility care due to the lack of treatment standards by implementing strict guidelines for the use of ICSI. This insurance agency covers approximately 90% of the patients undergoing In Vitro Fertilization (IVF) treatment in our program thereby providing access to many individuals who may not otherwise consider these services due to cost. Conversely, these guidelines may affect quality of patient care. This study investigated treatment outcomes during the 4 years following the implementation of these guidelines in women ≤40 years of age undergoing their first IVF cycle. Couples with and without a diagnosis of male factor infertility, according to the WHO laboratory manual (4th Edition), underwent IVF with or without the use of ICSI respectively.

Design: Retrospective cohort study in an academic IVF center.

Materials and Methods: Treatment cycles completed between January 2008 and December 2011 were reviewed after IRB approval. Ovarian stimulation was achieved using standard gonadotropin releasing hormone protocols. Oocytes were retrieved approximately 36 hours after human chorionic gonadotropin administration and inseminated 4 hours post retrieval with 150,000 motile sperm/milliliter (INSEM) or by injection of a single sperm (ICSI). Fertilization was assessed 16 to 18 hours later (day 1). Oocytes with two pronuclei were cultured until embryo grading on day 3 with transfer on day 3 or 5. Luteal support with progesterone continued until 8-10 weeks gestation for those patients with a positive pregnancy test.

Results: IVF cycles were analyzed according to method of insemination. There were no significant differences between the 2 groups with respect to mean age, BMI, ovarian reserve, days of stimulation, amount of medication given, number of mature oocytes or fertilization rate. Patients with inseminated oocytes had significantly more good quality embryos available on day 3 and significantly fewer embryos transferred than patients in the ICSI group. However, the clinical pregnancy and live birth rates were similar in both groups. Statistical analyses used T-test and Chi Square. $P<0.05$ is considered significant.

	ICSI	INSEM	*P* Value
Number of Patients / Cycles	86	76	-
Mean Age (years)	34	34	0.6409
Mean number of mature oocytes*	9.4	11.0	0.1410
Mean number of good quality embryos*	3.6	5.9	0.0018
Mean number of embryos transferred	1.9	1.6	0.0247
Clinical Pregnancy Rate / ET	41%	40%	0.9236
Live Birth Rate / ET	34%	34%	0.9919
Clinical Miscarriage Rate / ET	17%	15%	1.0000

*per cycle; ET - Embryo Transfer

Conclusion: These results show that in our center restricted use of ICSI did not lead to poorer treatment outcomes. In fact, standard insemination use in non-male factor patients yielded a significantly higher proportion of good quality embryos and fewer miscarriages. While ICSI is a valuable procedure, it is not essential for patients without male factor infertility.

Support: None.

EVALUATION OF AN ANTI-PNEUMOCOCCAL CAPSULAR POLYSACCHARIDE IgG ELISA FOR SCREENING IMMUNE RESPONSE TO PNEUMOCOCCAL POLYSACCHARIDES: SINGLEPLEX vs. MULTIPLEX ASSAY

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Background: Measurement and interpretation of immune response to pneumococcal capsular polysaccharides (PCP) are complicated due to the diversity of serotypes. Multiplex bead-based assays are usually employed in large reference laboratories for this purpose. These methods are technically complex and associated with high cost. We evaluated the usefulness of an ELISA test as a screening method by comparing results to a 14-plex bead-based assay.

Method: The total anti-PCP ELISA plates (The Binding Site Ltd, UK) are pre-coated with a mixture of the all PCP antigens contained in the 23-valent vaccine (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22, 22F, 23F and 33F). Samples were pre-absorbed with common cell wall polysaccharides to remove nonspecific antibodies. The xMAP pneumococcal 14 assay (Luminex Corporation, USA) simultaneously measures serotype-specific IgG levels against serotypes 1, 3, 4, 6B, 7F, 8, 9N, 9V, 12F, 14, 18C, 19A, 19F and 23F using polystyrene beads in combination with fluorescence detection. Analytical accuracy of the ELISA assay was evaluated using FDA reference serum 89SF. 20 pairs of pre- and post-vaccination sera and 56 single specimens from patients were used in the evaluation. Results of the multiplex assay were interpreted using a classification scheme that defines an adequate response as 70% of serotypes achieving levels greater than 1.3 μg/mL.

Results: Assay performance characteristics are summarized in Table 1. The total anti-PCP IgG ELISA showed acceptable precision, and analytical and clinical accuracy. A cutoff value was established for the total anti-PCP IgG ELISA using paired pre- and post-vaccination sera with a ratio (post/pre) greater than 2. Receiver operating characteristic analysis showed the area under curve (AUC) to be 0.957.

Conclusion: Classification of immune response to pneumococcal polysaccharide is similar when using either the total PCP ELISA or the multiplex bead-based assay. The anti-PCP ELISA may be a cost-effective alternative to multiplex assays for screening immune response to pneumococcal polysaccharide.

Table 1. Evaluation results of the total anti-PCP ELISA against the xMAP pneumo 14 assay

Precision (n=8)	Intra-assay CV: 1.4-2.5% Inter-assay CV:1.4-5.4%
Linear range	4.3-434.0 μg/mL
Analytical sensitivity (% recovery of FDA reference standard serum)	119.9%
Cutoff value	Protective: ≥143.5 μg/mL Non-protective : <143.5 μg/mL
Quantitative Correlation between anti-PCP ELISA and the sum of 14 serotyps from the Pneumo 14 assay (n=94)	Corr coef (R): 0.8520
Agreement on diagnosis classification of paired pre- and post-vaccination samples (Normal vs. low response) n=20	Overall agreement: 95.0% Normal response agreement: 100% Low response agreement: 80.0%
Agreement on diagnosis classification of individual patient sample (protective vs. non-protective) n=56	Overall agreement: 94.6% Protective agreement: 88.0% Non-protective: 100%

EXPERIENCES OF MANAGING LIQUID NITROGEN TANKS IN A LOW PATIENT VOLUME ACADEMIC INFERTILITY CLINIC

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Objective: The fertility clinics use liquid nitrogen (LN) tanks for storing sperm, oocyte, embryo and other reproductive tissues. Maintaining the frozen materials in the tank requires routine filling of the tank with LN. Failure or negligence can cause damage to the stored materials and thus may result in legal repercussions for the clinic. The number of LN tanks a clinic possesses depends on the patient volume it handles. The longevity of a tank usually depends on its makeup but can be influenced by how it is handled during use. Consumption of LN is not same for all tanks thus the refilling time of one tank can be different from that of the other. Proper management of the cryo tanks is hence an important task for the clinic personnel. In this report we share our experiences of managing LN tanks comparing their LN consumption pattern and factors that cause differential consumption.

Materials and Methods: The study dealt with cryo tanks of the same makeup (MVE) representing 4 models: XC-47/11, XC-34/18, SC-33/26 and APOL-SX35. There were 2 tanks in each of models XC-34/18 and XC-47/11, while the model SC-33/26 and APOL-SX35 contained 1 tank of each. Retrospective review was performed of the LN filling records of these tanks during the period of 2005 to 2010. Besides, in 2011 and 2012, the tanks were rearranged and relocated in a planned way and LN level in the tanks were recorded at defined time intervals. The objective was to see if the LN consumption varies with the season, storage location, and between empty and specimen filled tank. Relations between LN utilization and tank specific features were explored.

Results: The tank pool contained 7 to 23 yrs old 6 tanks. All tanks exhibited the manufacturer provided specification of LN consumption. In 17% cases the LN supplier failed to deliver LN on due delivery date but were always courteous informing the delay. In such circumstances resolution occurred within 24 to 48 hours. The supplier's containers had less amount of LN than expected in some summer deliveries. Reserve tank was advantageous for the smooth operation of the cryo unit. The refill time, considering 25 cm as set mark, was approximately 3 w, 5-6 w and 6-7 w for XC-47/11, XC-34/18 and SC-33/26 model tank, respectively. The specimen filled tank consumed more LN compared to empty tank. The differences in LN consumption by the tanks between summer and winter were not significant. The LN consumption by all tanks was high in location B compared to location A. The personal protective devices (face shield, gloves and O_2 monitor) and roller base supporting the tank found utterly necessary. Incidences documented were all related to personnel safety but were minor in nature.

Conclusion: 1) LN consumption by cryo tanks may vary in different locations of the same building if there is considerable variation in ventilation, light and temperature, 2) clinic specific calendar for LN order can be established based on previous LN consumption record, 3) LN emergency from unexpected delay of delivery can be avoided by establishing LN reserve, 4) any negligence in filling LN tanks is timely identifiable by proper record keeping and its supervisory review, 5) roller base is vital for the protection of the tank and also for operational safety.

INCREASE OF SPERM DNA FRAGMENTATION WITH AGE IN CLINICAL PATIENTS

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Introduction: SCSA data were obtained in a previous study on 97 healthy men ages 22−90 (Wyrobek et al PNAS 103:9601). 20's age men had a mean 20% DFI. The mean %DFI had incrementally increasing values reaching a clinical threshold of 30% DFI at age 56.9. 11/12 men above age 60 had >30% DFI. %DFI vs. age was significant ($r=0.72$ <0.01). A significant (decreasing) linear relationship ($P < 0.001$) was observed for %HDS vs. age.

Methods: n= 3044 men of couples seeking clinical infertility evaluation. Routine semen collection was done with instructed 2−5 days abstinence, and frozen aliquots were sent to our SCSA Diagnostic center.

Results: The mean and (maximum) values for % moderate DFI, % high DFI, % total DFI and % HDS were: 10.4 (67.2), 10.47 (69.20), 20.87 (96.8) and 11.3 (53.9) respectively. For each year of age increase, the average total %DFI increased by 0.6%. ($P < 0.001$). Total %DFI was relatively constant at (mean 17%) from age 22 to 38 and then increased significantly from age 39 to 80. The inverse prediction to estimate the ages associated with our current clinical threshold of 25% DFI for natural and IUI conception was age 45.5. Moderate and high %DFI increased in near equal values from age 25 to 58 at which point the %high DFI diverged to higher values. A significant (decreasing) linear relationship ($P < 0.001$) was observed for %HDS vs. age. All age patients had very significant variations. 29% of patients had values above the 25% DFI threshold for increased statistical risk for natural and IUI conceptions. 14/20 men above 50 yrs. age had > 25% DFI. 5% of men had a >50% DFI.

Conclusions: This is the first large study comparing SCSA parameters of 97 healthy non-patients to 3044 men of couples seeking infertility status. In both studies there were very significant increases over time with sperm DNA fragmentation likely due in large part to natural oxidative stress damage as related to the aging process. Patients were known to have medical factors such as varicocele, high fever, use of medications such as cortisone and SSRI's, toxicant exposure, infection, and diseases such as cancer and diabetes. The decrease in %HDS in both studies suggest that sperm from older men have a more mature sperm chromatin package including greater exclusion of histones. This may be related to a slowing of spermiogenesis steps in older men. The lower pregnancy success rate for men >50 years of age is likely due in part to damaged sperm DNA.

INVERSE RELATIONSHIP OF IVF/ET CYCLE VOLUME AND OUTCOMES: AN ANALYSIS OF DATA RELATED TO IN VITRO FERTILIZATION/EMBRYO TRANSFER CYCLES IN TEXAS DURING 2011

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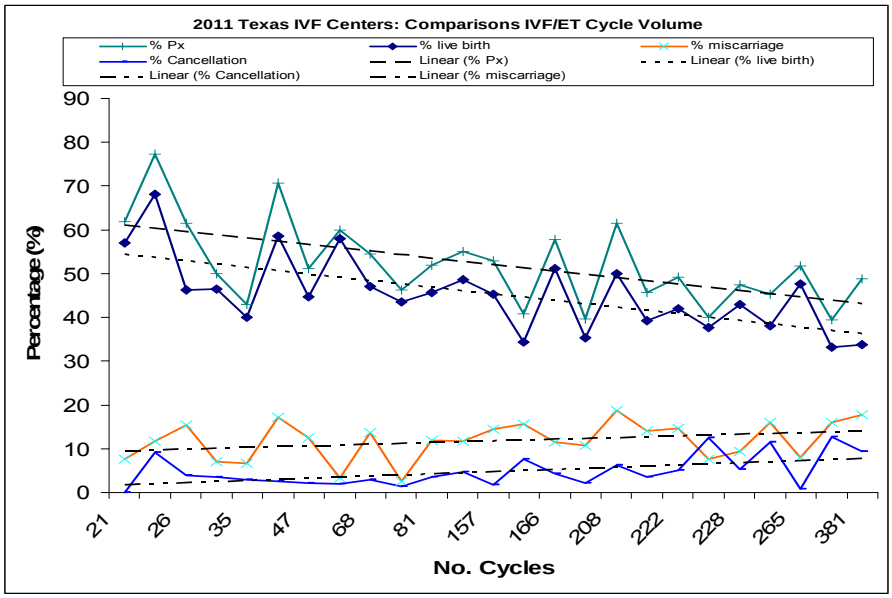
We have analyzed the data provided in the Society for Assisted Reproduction Technology (SART) Clinic Report for 2011 regarding patients 37 years of age and younger, which were treated utilizing IVF/ET in the State of Texas. Data analysis included clinics performing a minimum of 20 cycles upwards to a volume of 500 cycles per year.

In Texas, 37 centers performed IVF/ET in 2011. Out of these 37 centers, nine have reported no data to SART and three of the reporting centers performed either less than 20 or more than 500 cycles. Thus, leaving the data from 25 centers for the case outcome analysis (See Figure below). The center performing the most cycles performed 314 cycles and the center performing the fewest cycles reported data on 21 cycles. Data were analyzed using regression analysis and correlation analysis.

The volume of cycles performed by specific clinics was found to inversely correlate ($p<0.05$) with the likelihood of pregnancy resulting in a live birth. A positive correlation was found for the number of cycles performed and the occurrence of miscarriage ($p<0.05$). A positive correlation was demonstrated for volume of cycles performed and the likelihood of cycle cancellation ($p<0.05$).

Previous analysis has demonstrated that patient mix is responsible for only a minor part of pregnancy chance and other outcome measures found between centers that perform IVF/ET (Lintsen, et al, Hum Reprod 2010;25(1):110-117. The possibility that quantity of cycles of IVF/ET performed by a center might influence outcome is the subject of this current analysis. Regression and correlation analysis find that the likelihood of live birth is inversely related to the volume of cases performed in a specific center using SART annual report data analysis. In Texas in 2011 there was a positive correlation between IVF/ET cycle cancellation rate and the number of cycles a center performed and the likelihood of miscarriage, should pregnancy occur, was positively correlated with center cycle volume.

It is possible that the concept of "Diminishing Returns" is applicable to the performance of IVF/ET treatment.



PREGNANCY AFTER THE CALCIUM IONOPHORE ACTIVATION AND ANEUPLOID SCREENING USING a-CGH IN GLOBOZOOSPERMIA PATIENT

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Objective: To report a successful pregnancy after transfer of embryos derived from oocytes activated by calcium ionophore after intracytoplasmic sperm injection (ICSI) with round-headed sperms and aneuploid screening using array comprehensive genomic hybridization (a-CGH).

Design: Case report in a private IVF clinic.

Materials and Method(s): A 28-year-old patient and her 31-year-old husband, diagnosed with 100% globozoospermia, underwent ICSI, oocyte activation and chromosome screening using a-CGH. Ten metaphase II oocytes were injected with round-headed spermatozoa. After ICSI, the oocytes were treated with 10 µM calcium ionophore solution for 20 minutes at 37°C in 6% CO_2. The fertilization was checked 18 hours later. On the morning of day 3, one blastomere was biopsied from embryos which had at least 6 cells and sent to the genetic lab for aneuploid screening using a-CGH. Two euploid embryos were transferred on the fifth day after oocyte retrieval. Supernumerary normal embryos were vitrified for future use. Ongoing pregnancy rate was noted.

Result(s): This couple experienced only 12% fertilization after ICSI in their first cycle. On the second cycle, 8 out of 10 metaphase II oocytes were fertilized after ICSI and calcium ionophore activation immediately after ICSI. 5 out of 8 embryos were diagnosed as euploid. Two normal blastocysts were chosen for transfer. Clinical pregnancy was confirmed at 7 weeks of gestation with two heartbeats. Two healthy babies were born in July 2012.

Conclusion (s): Artificial oocyte activation using calcium ionophore is beneficial in patients with globozoospermia. This study showed that the method of oocyte activation does not affect chromosome constitution or the normal growth of pre-implantation embryos. Further studies are needed to confirm the safety of oocyte activation.

Key Words: globozoospermia, round-headed sperm, calcium ionophore, oocyte activation.

TEMPERATURE VARIATIONS WITHIN AND BETWEEN INCUBATORS

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There are many variables to take into consideration to have a successful assisted reproduction technology (ART) program. One such variable is temperature. Numerous studies have been conducted to investigate the effect temperature has on embryo development and pregnancy rates. Because temperature has a direct impact on embryo homeostasis, it is important to monitor and control temperatures in the ART laboratory. The aim of this study is to investigate temperature differentials inside front loading incubators typically used in ART laboratories. With the use of wireless temperature probes, temperature between the front and back of the incubator as well as temperature between and among the shelves are evaluated. This was a prospective, experimental trial with external controls that was conducted at an ART laboratory in a tertiary-care, university hospital. The same make and model incubators had significantly different temperature readings even though they were both set to 37.0^{o}C. There were significant temperature differences among top, middle and bottom shelves as well as significant temperature differences between the fronts and backs of shelves. There were temperature differences within and between our front-loading incubators. Thus, laboratory personnel should evaluate their incubators and determine if there are differences within and between their incubators and if so, what, if anything, should be done to correct these discrepancies.

TEMPERATURE VARIATIONS WITHIN IVF INCUBATORS AS A FUNCTION OF LOCATION AND DEVICE

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Introduction: In the laboratory, a stable culture environment is imperative for embryo development. In any incubator, whether large or small box-type incubators or desk-top models, door openings may perturb the internal environment to the extent that embryo development is compromised. In order to accurately assess temperature variability throughout an incubator and within smaller chambers placed in the incubator, a variety of temperature measuring devices were employed and temperatures monitored over time.

Methods and Materials: Single-point temperature measurements were taken using four fine-wire Greissinger thermocouples (Origio; Mt. Laurel, NJ) placed in an oil dish. The four thermocouples were fed through the inner door and affixed to the outside of the incubator so temperatures could be taken without opening either the outer or inner doors of the incubator. These probes had been calibrated against an NBS thermometer. Continuous measurements were taken using either multiple small data loggers (micro-DL) with a short thermocouple (Marathon Products; San Leandro, CA) that could be submersed in oil, or multiple wireless data loggers (wireless DL; Marathon Products). Temperature readings were taken once every 2-6 sec throughout all studies with the micro-DL or the wireless DL. Microchambers (Digital Medical Instruments; Tonawanda, NY) were used to determine stability in a closed environment within an incubator. Temperature deviations were assessed within a Hera Cell incubator (Thermo Fisher Scientific; Waltham, MA) with three shelves and an inner divided door.

Results: Exp 1: There was a range of temperature variability across identical devices from the same manufacturer when placed in the same location (middle of bottom shelf) within an incubator (Greissinger=±0.2^{o}C, micro-DL=±0.2^{o}C, wireless DL=±0.9^{o}C.) Because of this variability, only the micro-DL was used in the rest of the experiments to monitor continuous temperature outcomes. **Exp 2:** Average temperatures, as determined using 5 probes on each of three shelves, were 0.45^{o}C different across the shelves, with the hottest shelf being the upper shelf (37.34^{o}C) and the coolest being the bottom shelf (36.89^{o}C). Temperatures of multiple probes placed around a given shelf varied by as much as 0.7^{o}C per shelf. **Exp 3:** A fresh oil dish at room temperature (22-25^{o}C), placed into an incubator, took a minimum of 4 hr to reach a temperature within 0.3^{o}C of the average for the shelf and another 5 hours to finally reach a temperature that did not vary by more than 0.1^{o}C from the average for the remainder of the incubation time (14-18 hr). **Exp 4:** Once the oil dish had been equilibrated overnight, the outer door and one of the three inner doors were opened for 5 sec to simulate removal of a dish from the incubator, but the dish was left in place on the shelf. The temperature in the oil dish decreased by 4^{o}C within 20 sec after opening. This temperature stabilized 3 min later at a temperature that was 0.1^{o}C cooler than the overnight equilibration temperature. A second door opening 1.5 hr later dropped the temperature of the oil dish 3.1^{o}C in 30 sec. After this door opening, the oil returned within 15 min to a temp that was 0.3^{o}C cooler than the equilibration temp; after one hr the temperature had still not returned to the equilibration temperature. **Exp 5:** Temperature variation was significantly lower within the Microchamber after door openings. After the first door opening, temperature in the oil dish decreased by 0.1^{o}C within the first 2 min and then remained at this temperature until the second door opening. After the second door opening the temperature decreased by another 0.1^{o}C and stayed there until the termination of the experiment.

Conclusions: Performance of temperature devices was highly variable between identical devices from one manufacturer and among devices from different manufacturers. Temperature was not consistent between different locations on a shelf or between shelves within an incubator. Furthermore, the temperature of an oil dish in an incubator dropped very quickly, but took several hours to recover, after even a short door opening. Multiple door openings of an incubator prevented stabilization and the return of oil temperatures to that found after extended overnight equilibration. However, the use of small, sealed chambers within an incubator stabilized these fluctuations.

Conflict of Interest Disclaimer: No author has a financial interest in any of the products used in this study.

THE HEAT IS ON: ROOM TEMPERATURE AFFECTS LABORATORY EQUIPMENT

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Temperature is an essential component of cellular physiology and a critical aspect of embryo culture. It is well documented in the literature that temperature stability is necessary during in vitro fertilization. Because temperature can influence in vitro reproduction, temperature control is vital in assisted reproduction to ensure that no gametes or embryos are exposed to extreme temperature fluctuations. The objective of this study was to evaluate the effect of ambient room temperature on equipment typically used in vitro fertilization (IVF). We set the control temperature of the room to 20°C (+/-0.3) and used wireless temperature probes to record temperatures of the following equipment: six microscope heating stages, four incubators, five slide warmers and three heating blocks. We then increased the room temperature to 26°C (+/-0.3) or decreased it to 17°C (+/-0.3) and monitored the same equipment again. We wanted to determine what role, if any, changing room temperature had on equipment temperature fluctuation. There was a direct relationship between room temperature and equipment temperature stability. When room temperature increased or decreased, equipment temperature reacted in a corresponding manner. Statistical differences between equipment were found when the room temperature changed. What is also noteworthy is that temperature of equipment responded within 5 minutes to a change in room temperature. Clearly, it is necessary to be aware of the effect of room temperature on equipment when performing assisted reproductive procedures. Room and equipment temperatures should be monitored faithfully and adjusted as frequently as needed, so that consistent culture conditions can be maintained. If more stringent temperature control can be achieved, human assisted reproduction success rates may improve.

THE HUNT FOR A METHOD TO PREDICT PREGNANCY OUTCOME BASED ON SPERMATOZOAL PARAMETERS – A CONTINUING SAGA

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We continue to look for variables in semen specimens that may act as discriminators to give us a method to predict pregnancy in couples seeking fertility treatments. From a database of 127 semen specimens taken from a university-based hospital infertility practice, we examined various sperm kinematics to determine their correlation to predicting pregnancy. The three main kinematics were average path velocity, straight line velocity, and curvilinear. We used several methods to determine if a relationship existed for kinematics and pregnancy. The first method attempted to identify simple kinematic values obtained following sperm separation via a gradient. This method failed to identify any kinematic that was highly predictive of a positive clinical pregnancy when intrauterine insemination was used with the post-gradient specimen. The second method used a statistical approach. A linear model was defined for the probability of pregnancy as a function of the variables and a Stepwise Logistic Regression Variable Selection was used to determine the variables that had statistically significant contributions to the model, but this model only yielded 4% accuracy for predicting a pregnancy. The third method was also a statistical approach. Data mining techniques were used to split values of the sperm characteristic variables into low and high, and then determine the right combination that would result in a decision tree for pregnancy outcome. This method yielded 48% accuracy in predicting which patients would become pregnant after intrauterine insemination with the specific spermatozoal specimen. We continue to investigate methods to develop an even better prediction function, possibly using a combination of the aforementioned methods.

THE POTENTIAL BENEFIT OF PREIMPLANTATION ANEUPLOIDY TESTING USING a-CGH FOR IVF PATIENTS

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Objective: To assess the potential benefit of pre-implantation aneuploidy testing on the outcome of in vitro fertilization (IVF) patient.

Design: Retrospective study performed in a private IVF clinic.

Materials and Methods:

IVF patients from Jan 2010 to December 2011 were retrospectively evaluated for various outcomes. Women included in the study had to meet the following criteria: between the age of 25-45, FSH on day 3 < 18, BMI< 40, the presence of four or more embryos with ≥ 6 cells on day 3. Typically, one cell per embryo was biopsied on day 3 of development, and pre-implantation genetic using a-CGH (array Comprehensive Genomic Screening) or FISH (Fluorescent In Situ Hybridization) was performed follow by routine blastocyst transfer. End points include the rates of pregnancy, implantation, spontaneous abortion, and transfer cancellation due to aneuploidy.

Results: An interim analysis was conducted on 40 cycles using a-CGH. The average age was 36.6 ± 5.4 and the average of embryos biopsied on day 3 was 7.3 ± 2.8 per patient. From 284 embryos were biopsied, 94 (33.1%) were found euploid with the blastocyst formation of 70% (66/94), and 168 (59.2%) were aneuploidy with 19.6% (33/168) blastocyst formation. 22 samples were not able to analyze (7.8%). The overall pregnancy rate per embryo transfer is 51.8% (14/27). The pregnancy rate per ET for patient in 2 age groups with a-CGH (>40 and 38-40) was 50%, and 40% respectively compared to15.8%, and 20% without a-CGH.

Conclusion: Initial results for patients of advanced maternal age (>38 years) and repeated implantation failure were encouraging. The cancellation rate is increased with a-CGH group (with age > 38). However, further study is required to confirm whether or not embryos screening using a-CGH are beneficial.

Support: Red Rock Fertility Center

THE USE OF ACCU-BEADS AS A QUALITY CONTROL FOR THE COMPUTER AUTOMATED SEMEN ANALYZER

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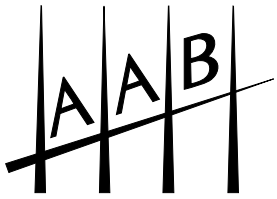
The ability to verify the accuracy and precision of a diagnostic instrument is essential, especially in the medical field. The computer automated semen analyzer (CASA) is no exception. Though CASA has proven to be accurate in a specified range of sperm concentrations, imprecision errors can arise from various sources. The purpose of this study is to determine the precision and accuracy of Accu-Beads and their utility as a quality control (QC) product for CASA. This is a retrospective observational study conducted at an Assisted Reproductive Technology laboratory in a tertiary-care, university hospital. We found that the means ± 1SD in M/mL for the two vials with different concentrations of Accu-Beads analyzed by two distinct methods were as follows: Vial 1 CASA, 42.7 ± 3.48; Vial 1 Manual, 42.6 ± 3.45; Vial 2 CASA, 22.8 ± 2.21; and Vial 2 Manual, 22.9 ± 2.81. The CASA counts did not vary significantly from the manual counts for Vial 1 or for Vial 2. The bead concentrations listed by the manufacturer for each vial were below the 95% confidence interval for the values we obtained. In conclusion, Accu-Beads meet enough of the requirements of a good control material to be acceptable for daily QC use, especially if we set our own ranges of acceptability for each vial of Accu-Beads.



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