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May 15-17, 2008
Flamingo Hotel
Las Vegas, Nevada

Oral Abstract Presentations
Friday, May 16, 2008
12:45-2:45 p.m.

Poster Session
Friday, May 16, 2008
6:15-8:15 p.m.

AAB Conference/CRB Symposium
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Alphabetical Listing By Title

A COMPARISON OF DNA DAMAGE BETWEEN VITRIFIED AND SLOW-COOLED MOUSE EMBRYOS

J.E. Graves-Herring, ETSU Fertility Services, Johnson City, Tennessee;
W.R. Boone, PhD, HCLD/ELD(ABB), Greenville Hospital System,
University Medical Center, Greenville, South Carolina

COMPARISON OF TYGERBERG STRICT AND WHO 4TH EDITION SPERM MORPHOLOGY ASSESSMENTS: ANALYSIS OF 7 YEARS OF AAB PROFICIENCY TESTING DATA

Erika Jensen, MS[1], Euijung Ryu, PhD[2], Sandra Bryant, MS[2],
Dean Morbeck, PhD, HCLD(ABB),[1]
Mayo Clinic, Department of Laboratory Medicine and Pathology[1] and Division of Biostatistics[2], Rochester, Minnesota

ELECTIVE SINGLE EMBRYO TRANSFER (E-SET) REDUCES MULTIPLE PREGNANCIES WITH INCREASED IMPLANTATION RATES WITHOUT REDUCING LIVE BIRTH RATES IN GOOD PROGNOSIS PATIENTS

Christine Briton-Jones, PhD, HCLD(ABB), Nicole Buehler, Hal Danzer,
Mark Surrey and David L. Hill, PhD, HCLD/ELD(ABB), Southern California Reproductive Center and ART Reproductive Center, Beverly Hills, California

IN VITRO CULTURE OF HUMAN OOCYTES AND EMBRYOS: THE EFFECT OF OXYGEN TENSION ON RESULTING OFFSPRING

William R. Boone, PhD, HCLD/ELD(ABB)[a], M. Darcy Herlong,[b]
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PARTHENOGENIC ACTIVATION AS AN ASSESSMENT OF CRYOSURVIVAL AND EARLY DEVELOPMENTAL COMPETENCE OF HUMAN OOCYTES

David L. Walker, MSc, ELD/TS(ABB), Jolene R. Fredrickson, MS, Kathrynne M. Barud, BS, TS(ABB), Dean E. Morbeck, PhD, HCLD(ABB), Reproductive Endocrinology and Infertility, Dept. of Obstetrics and Gynecology, Mayo Clinic College of Medicine, Rochester, Minnesota

REAL-TIME PH PROFILING OF IVF CULTURE MEDIUM USING AN INCUBATOR DEVICE WITH CONTINUOUS MONITORING

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VITRIFICATION AND WARMING OF BLASTOCYSTS PRODUCES HIGH IMPLANTATION AND PREGNANCY RATES WHEN COMPARED TO SLOW FREEZING

Best Oral Abstract

Jean Popwell, PhD, HCLD(ABB), Erin Fischer, BS, Elizabeth Holmes, BA, Mariluz Branch, MS, TS(ABB), Carolyn Givens, MD, Carl Herbert, MD and Joe Conaghan, PhD, HCLD/ELD(ABB)
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VITRIFICATION AND WARMING OF OOCYTES MAY BE IMPACTED BY THE NUMBER OF OOCYTES RETRIEVED

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A COMPARISON OF DNA DAMAGE BETWEEN VITRIFIED AND SLOW-COOLED MOUSE EMBRYOS

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The objective of this study is to determine if there is more DNA damage caused by freezing with vitrification or a slow-cooled method. This was a randomized, prospective, pilot study with two-cell mouse embryos (B6C3F1). Vitrified embryos were exposed to a holding solution then a vitrification solution that consisted of Dulbecco's Phosphate Buffered Saline without calcium and magnesium, ethylene glycol and dimethyl sulfoxide. After exposure to the vitrification solution, embryos were aspirated into Stripper Tips®. Tips were sealed on both ends, plunged into liquid nitrogen (LN_2) and stored. At a later date, the tips were thawed and embryos were rehydrated and then cultured at 36.7°C and 5% CO_2 and air for 24 h in 50 µL drops of Human Tubal Fluid (HTF) overlaid with oil. Slow-cooled embryos were exposed to a series of three solutions that used Dulbecco's Phosphate Buffer Solution as a base and contained varying concentrations of human donor serum, propandiol and sucrose. After embryos were exposed to the solutions, they were inserted into straws that were heat-sealed at both ends and frozen in a Planer Freezer. Following freezing, straws were removed, placed in LN_2 and stored. At a later date, the straws were thawed, rehydrated and moved to culture as described above.

This study had three controls: controls (cultured in HTF), positive controls (cultured in HTF then exposed to UV light) and fresh controls (collected from females at the eight-cell stage). All five groups of embryos were exposed to the Comet Assay (Trevigen®) and evaluated for comet tails. A confidence interval was determined for the controls and the upper bound limit was used as a normal tail length to differentiate normal tails from abnormal tails in all groups. Chi-square analyses were performed for the length of comet tails. The only significant differences observed when each group was compared to another were when the fresh controls (37/41=90%) were compared to controls (24/54=56%), positive controls (23/56=59%), vitrified (27/58=53% [$P<0.001$]) and slow-cooled (13/32=59% [$P=0.002$]) groups.

In conclusion, there is no significant difference in DNA damage between vitrified and slow-cooled two-cell mouse embryos. The significant difference observed in fresh embryos compared to the other groups is concerning because there appears to be DNA damage caused by culture of embryos.

COMPARISON OF TYGERBERG STRICT AND WHO 4TH EDITION SPERM MORPHOLOGY ASSESSMENTS: ANALYSIS OF 7 YEARS OF AAB PROFICIENCY TESTING DATA

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In 1999, the 4th edition of the WHO laboratory manual for semen analysis incorporated strict criteria for morphology assessment. At the time, methods used for analysis included but were not limited to Tygerberg strict, WHO 2nd and WHO 3rd editions. Since 1996, quality control of semen analysis has been facilitated via a proficiency testing service (PT) established by the American Association of Bioanalysts (AAB). In the current study, only data from laboratories that submitted results for 10 or more events were analyzed. Three hundred twenty-three laboratories fulfilled these criteria, submitting a total of 11,445 PT challenges. The majority of laboratories reported their results using Tygerberg strict (66.6%), WHO 3rd (20.9%) or WHO 4th (8.7%) methods. The most common staining method used was Diff-Quik® (46.0%). The goal of this study is to compare results from labs reporting WHO 4th morphologies versus labs reporting Tygerberg strict criteria. Generalized linear mixed models with a gamma distribution, a log link and random laboratory effects were used to determine if morphology analysis varied significantly over time based on method, stain and significant interactions. All results are represented as predicted morphology, since different scoring procedures have different slopes over time. In addition, different stains have different morphology intercepts. In this model, stain, methods and time were all highly significant factors ($p<.0004$) along with significantly different slopes for each method over time ($p<.0001$). The following shows the median predicted morphology (% normal) for sperm stained with Diff-Quik® for WHO 4th versus Tygerberg strict criteria:

Method	Predicted Median (IQR)*	Predicted Median (IQR)*
Date	May, 2001	December, 2007
WHO 4th Edition	27.6 (24.9, 35.8)	16.9 (13.0, 19.9)
Tygerberg strict	9.6 (8.4%, 10.6%)	7.5 (6.7, 8.6)

Overall, the AAB PT challenges showed a wide variation among laboratories. Those laboratories using WHO 4th edition and Diff-Quik® staining had the greatest decrease in morphology results with time. Labs using the same stain and Tygerberg strict criteria showed a smaller, though significant, decrease over time. In general, Tygerberg strict values were significantly lower than those of WHO 4th during this time period ($p<.0001$). In summary, while WHO 4th and Tygerberg strict are based on the same methods, laboratories performing PT with WHO 4th criteria obtained morphology values 2 to 3 times higher than labs reporting results with Tygerberg strict criteria. These results could have significant clinical implications and warrant further analysis.

*IQR=interquartile range

ELECTIVE SINGLE EMBRYO TRANSFER (e-SET) REDUCES MULTIPLE PREGNANCIES WITH INCREASED IMPLANTATION RATES WITHOUT REDUCING LIVE BIRTH RATES IN GOOD PROGNOSIS PATIENTS

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Background and Significance: The United States of America falls behind parts of the world such as, Europe and Australia, when it comes to taking steps to reduce the incidence of multiple pregnancies associated with assisted reproductive treatment (ART).The most common reason given for this is the high patient costs of ART per cycle in the USA. It is widely held that e-SET cycles will not hold the same chance for live birth as multiple embryo transfer cycles. The literature originating from countries that provide significant funding towards ART treatment challenges this widely held belief. This study describes outcomes of implementing e-SET in good prognosis patients.

Objective: This case cohort study presents the use of e-SET in a private ART clinic in the USA.

Materials and Methods: Patients that obtained two or more blastocysts at grade 5BB (Gardner) or better quality on day five of embryo culture, where the embryos were derived from oocytes from a woman younger than 35 years old or had been shown to be euploid following PGD-AS were offered e-SET. The non-parametric Mann Whitney test was used to determine statistical significance.

Results: 93 treatment cycles were offered e-SET. 54/93 (58%) accepted e-SET with 44/54 achieving pregnancy (82%) while 39/93 (42%) chose to transfer two embryos (e-DET) with 30/39 achieving pregnancy (77%). e-SET led to significantly higher implantation rates than e-DET, where $P< 0.01$. 41/54 (76%) of E-SET were transferred to women over 41 years of age but using donor oocytes. All pregnancies following e-SET were singleton. Pregnancies following e-DET: 1/30 (3.3%) was a triplet pregnancy, 11/30 (36%) were twin pregnancies and 18/30 (60%) were singleton pregnancies.

Conclusion: e-SET leads to high pregnancy rates with very low risk of multiple pregnancies. The success of e-SET is not reduced when embryos are being transferred into women of advanced maternal age. Of interest is that e-SET in this study was associated with significantly higher implantation rates.

IN VITRO CULTURE OF HUMAN OOCYTES AND EMBRYOS: THE EFFECT OF OXYGEN TENSION ON RESULTING OFFSPRING

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Objective: Cattle and sheep embryos cultured under high oxygen tension (20% O_2) and then transferred into recipient females produce live offspring with increased birth weights. The purpose of our study is to determine if a relationship exists between two oxygen tensions for human *in vitro* cultured embryos and subsequent infant birth outcome.

Design: Retrospective cohort study.

Setting: Tertiary care and infertility practice.

Patient(s): Female members of infertile couples (277) who underwent an Assisted Reproductive Technology (ART) procedure from January 1997 through December 2006 and subsequently gave birth to an infant.

Intervention(s): None

Main Outcome Measure(s): Birth weight of the infants.

Materials and Method: *In vitro* production of Day 3 human embryos was conducted in multi-gas incubators (Thermo Electron Corporation, Marietta, Ohio). The gas incubators used in this study were set at 20% O_2 (95% air, 5% CO_2) or 5% O_2 (88% N_2, 7% CO_2, 5% O_2). During the years included in this retrospective study, we used different types of culture media. Human Tubal Fluid (HTF) was used 62% of the time, while a combination of HTF with G-series media or HTF with in-house prepared media was used 38% of the time. Infant weight was determined at the time of delivery. In addition, infants were screened for the presence of birth anomalies after delivery and any anomalies found were recorded.

Result(s): There were 197 live, singleton births from embryos incubated in an atmosphere of 5% O_2 and 80 live singleton births from embryos incubated in an atmosphere of 20% O_2. The mean birth weight of singleton baby boys (n = 95) derived from embryos exposed to 5% O_2 was 3335.2 ± 575.9 g, while singleton baby boys (n = 40) derived from embryos cultured in 20 % O_2 averaged 3314.2 ± 689.2 g at birth. The mean birth weight of singleton baby girls (n = 102) developed from embryos exposed to 5 % O_2 was 3240.8 ± 543.6 g, while singleton baby girls (n = 40) developed from embryos exposed to 20 % O_2 averaged 3138.4 ± 670.2 g at birth. Average weight for all live singleton births (n = 197) from embryos incubated in 5 % O_2 was 3286.3 ± 600.0 g, while the average weight for live singleton births (n = 80) from embryos incubated in 20 % O_2 was 3226.3 ± 681.4 g. No differences were noted in the frequency of birth anomalies between incubator oxygen environments.

Conclusion(s): These data suggest that birth weight and anomalies among infants derived from *in vitro* production are not influenced by incubator environment settings of 5 % or 20 % O_2.

Key Words: Assisted Reproductive Technology, ART, birth weight, oxygen tension

PARTHENOGENIC ACTIVATION AS AN ASSESMENT OF CRYOSURVIVAL AND EARLY DEVELOPMENTAL COMPETANCE OF HUMAN OOCYTES

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Oocyte cryopreservation is considered an important method for fertility preservation. Like many new technologies, it is important to demonstrate mastery of the technique prior to implementation into clinical practice. While controlled rate freezing is commonly used by IVF laboratories, vitrification is technically different and requires demonstration of proficiency prior to implementation. The present study used oocytes immature at retrieval to compare freezing methods and establish a baseline for training and proficiency assessment. Both survival and parthenogenic activation were used as endpoints for analysis. Human oocytes were subjected to an activation procedure to aide in the assessment of oocyte competence following cryostorage by Controlled-Rate Freeing (CRF) and Vitrification (V). Twenty-three patients undergoing IVF consented to research with donation of immature/non-inseminated oocytes. The oocytes were randomized to three treatments; fresh controls, CRF or V, following maturation to the metaphase 2 stage. Both fresh and surviving cryostored oocytes were subjected to Ionomycin and a protein kinase inhibitor, 6-dimethylaminopurine (6-DMAP) 3 hours post-thaw. Oocytes were assessed for pronuclear formation and early cleavage development. Survival between the two treatments was not different. However, there was a trend for higher activation rates, as assessed by PN formation, in vitrified oocytes compared to CR frozen oocytes. The reciprocal was true for early development, with a high cleavage rate noted in activated CR frozen oocytes compared to activated vitrified oocytes. Although numbers of oocytes used in this preliminary work is low, it appears that parthenogenic activation may be a reliable method to assess cryosurvival and early developmental competence in human oocytes. Treatment Post Thaw Survival Pronuclear Formation Early Cleavage Fresh Controls --- 6/12 (50%) 5/6 (83.3%) Controlled-Rate 22/28 (78.6%) 14/22 (63.6%) 14/14 (100%) Vitrification 15/20 (75.0%) 15/15 (100%) 6/15 (40%)

REAL-TIME pH PROFILING OF IVF CULTURE MEDIUM USING AN INCUBATOR DEVICE WITH CONTINUOUS MONITORING

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Introduction:

The maintenance and monitoring of culture medium pH is a critical component of laboratory quality control during in vitro fertilization (IVF). Medium pH can not easily be measured under actual working conditions where the medium is often used in small drops under oil. In addition, the measurement of pH usually requires that the medium be removed from the incubator environment, and testing is slow and involves relatively large medium samples in which a pH electrode can be fully immersed. Under these circumstances, it can be difficult to accurately measure the working pH of the medium.

Recently, a new pH measuring device has become available that allows in situ measurement of pH in small droplets of medium that are identical to those used for oocyte and embryo culture. The device can monitor pH in real time and take continuous measurements at user specified intervals and without having to open the incubator door. Once the device is set up, it can be monitored remotely and it can be used to test a variety of culture environments.

Objective:

This study was designed to examine the pH equilibration profile of embryo culture medium within an incubator under standard culture conditions.

Materials and Methods:

A pHOnline "fluorescent decay time" pH meter (*MTG Altdorf, Germany*) was used to provide 'real time', *in situ* measurement of medium pH under actual culture conditions. The device utilizes a standard Nunc 4-well dish in which one well is fitted with a pH reactive disk (sensor dish). The sensor dish is prepared as for normal culture, depending on the users preferred technique. However, the dish must contain at least sufficient medium to completely cover the sensor spot.

In the first experiment, the medium to be tested (*EmbryoAssist, MediCult, Denmark*) was prepared as a single 50µl micro-drop overlaid with 1ml of pre-washed, non-equilibrated paraffin oil (*MediCult*). The micro-drop-sensor dish assembly was equilibrated in an incubator (*HeraCell, 150*), set to 37^{o}C with an environment of 6% CO_2 in air. Simultaneously, two 15ml centrifuge tubes with caps loosely in place, containing 3ml of culture medium, were placed within the incubator adjacent to the micro-drop-sensor dish assembly to act as controls. Further tests were conducted with sensor dishes containing 500µl of medium with or without a 500µl paraffin oil overlay, and 1 ml of medium without oil. All test assemblies were allowed to incubate for a minimum of 23 hours. The pH of the medium was logged at 5 minute intervals throughout. All samples were prepared at room temp (~20^{o}C) prior to placement in the incubator

Results:
The pH of the culture medium typically reached a stable pH of 7.33 ± 0.02 in the sensor dish assembly after a period of incubation in the gas controlled environment. The control tubes of culture medium showed the same pH as the sensor dishes when measured with a standard external pH meter (Beckman Coulter 300 Series). Repeat testing verified these results and the ability of the sensor dish to accurately measure pH.

Dishes containing 50µl of medium under oil took just over 8 hours to fully equilibrate, but once equilibrated, the pH remained relatively stable even when the dish was removed from the incubator for 1, 5 or 10 minutes. Dishes containing 500µl of medium under oil behaved similarly but took slightly longer to equilibrate.

Dishes that were prepared with 0.5 or1ml of culture medium, and without an oil overlay, equilibrated in under 1 hour. However, without the oil overlay, the pH of the medium began to rise immediately when these dishes were removed from the incubator. This effect was most pronounced in the dish with 0.5 ml of medium where the pH rose above 7.40 after 5 minutes under ambient conditions.

Discussion:
The sensor dish assembly appeared to be a reliable method for measuring pH when compared to the traditional method of measuring pH with a conventional meter. No significant differences were seen in pH readings between systems.

The time taken to equilibrate medium varied depending on the volume of medium used and the use of an oil overlay. Medium equilibrated quickly without an oil overlay, but very slowly with an oil overlay. The oil also appeared to act as an effective buffer maintaining pH for up to 10 minutes when the dishes were removed from the incubator. Dishes without an oil overlay showed dramatic pH changes when outside the incubator for a short time.

Conclusion:
Even with relatively small volumes of medium a minimum of 8 hours equilibration is needed to establish a stable pH under normal culture conditions. This suggests that a dish of culture medium with an oil overlay cannot be prepared for use the same day. However, once equilibrated, oil acts as a reliable buffer preventing fluctuations in pH during short excursions from the incubator.

VITRIFICATION AND WARMING OF BLASTOCYSTS PRODUCES HIGH IMPLANTATION AND PREGNANCY RATES WHEN COMPARED TO SLOW FREEZING

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Introduction:
Vitrification is a promising technology for blastocyst preservation following in vitro fertilization (IVF). Preservation of blastocysts is a routine part of IVF treatment and has traditionally been accomplished using slow freezing with low concentrations of cryoprotectants. Vitrification has recently emerged as an alternative procedure in the preservation of oocytes and embryos and it may confer some time saving and survival advantages over the traditional procedure for blastocyst cryopreservation.

Objective:
This study evaluates the efficiency of a blastocyst vitrification program when compared to a slow freezing program.

Materials and Methods:
Blastocysts remaining after transfer were slow frozen or vitrified on day 5 and/or 6 post retrieval. Blastocysts at all stages of development (early, expanding, expanded and hatching) were slow frozen using a kit (Sage/CooperSurgical, Trumbull, CT) or vitrified using a kit (Irvine Scientific, Santa Ana, CA) and stored individually in ¼ cc straws or cryotips immersed in liquid nitrogen. No artificial collapsing of blastocysts or other manipulations were performed during the procedure. Blastocysts were thawed or warmed on the equivalent of Day 4 in either a natural or controlled cycle and transferred after a short incubation. Pregnancy testing was performed 10 days later.

Results: The study evaluated 129 cycles of slow freezing with 259 embryos transferred and 65 cycles of vitrification and warming with 138 embryos transferred from January 2007 to the present.

	Slow freeze	Vitrification	P value
Cycles	129	65	
Embryos transferred (mean/patient)	259 (2)	138 (2.1)	NS
Pregnancies	33	39	
# sacs	36	52	
Clinical pregnancy rate (%)	25.6	60	P<0.005
Implantation rate (%)	13.9	37.5	P<0.000002

Discussion:
Vitrified embryos survive and implant at high rates after warming and transfer when compared to slow-frozen embryos. Although vitrification is technically a more challenging procedure, the benefits of the technology are immediately apparent. Vitrification has emerged as a viable alternative to slow freezing for human embryos at the blastocyst stage.

VITRIFICATION AND WARMING OF OOCYTES MAY BE IMPACTED BY THE NUMBER OF OOCYTES RETRIEVED

Joe Conaghan, PhD, HCLD/ELD(ABB), Elizabeth Holmes, BA, Erin Fischer, BS,
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Introduction:
Vitrification has been used successfully for the preservation of human oocytes. This technology uses high cooling rates and high concentrations of cryoprotectants to overcome the problems of ice formation and sensitivity to temperature and ionic balance that have made oocytes so difficult to freeze reliably.

Objective:
Using oocytes of the highest quality, this study assessed the differences in survival rates between donors with moderate and high numbers of oocytes.

Materials and Methods:
Five oocyte donors were recruited to participate in the study. All 5 had a history of oocyte donation where high numbers of oocytes were retrieved, good embryo quality was achieved and 1 or more pregnancies resulted. Following controlled ovarian hyperstimulation, oocytes were retrieved from each donor and all oocytes were vitrified within 2 hours from the time of retrieval. Cumulus cells were removed from the oocytes which were then vitrified in cryotips using cryopreservatives from Irvine Scientific (Santa Ana, CA). Each cryotip was loaded with a single oocyte and stored in liquid N_2 until warming. Recipients typically received 4-8 oocytes from one donor, which were warmed and subjected to ICSI 4 hours later. Transfer of resulting embryos was performed on day 3 or 5 post warming.

Results:
Two of the donors had very high oocyte numbers (35 and 37) and these oocytes did not tolerate the vitrification procedure (see table, donors 1 &3). The other 3 donors yielded oocytes that performed well after warming and have to date established 7 pregnancies in 8 recipient cycles, with 5 oocytes remaining in storage (enough for one more recipient cycle). For the 3 donors with lower oocyte numbers (donors 3, 4, & 5) the number of implantations per embryo transferred (9/15: 60%) is comparable to that seen in fresh cycles.

Donor #	Oocytes	Warming cycles	Pregnancy outcome
1	35	1	No pregnancy
2	37	1	No ET
3	18	2	1 singleton delivered, 1 SAB
4	19	3	1 singleton ongoing, 1 twin ongoing
5	23	3	1 twin ongoing, 1 ectopic, 1 SAB

Discussion:
In this small study, donors with proven fertility were used to test a vitrification system. Oocytes that came from large cohorts seemed overly sensitive to the procedure and outcomes were poor. However, with more moderate oocyte numbers, implantation and pregnancy rates were acceptable for this patient population. The data suggest that an oocyte bank could be established using young donors with modest oocyte numbers.

AAB Conference/CRB Symposium
Poster Session
Friday, May 16, 2008
6:15-8:15 p.m.
Flamingo Hotel, Las Vegas, Nevada

1	**COMPARISON OF THE MANUAL, CASA, AND SCA METHODS FOR SEMEN ANALYSIS REPORTING** **J. Glenn Proctor, Jr., MHA,** William R. Boone, PhD, HCLD/ELD(ABB), H. Lee Higdon, III, PhD, HCLD(ABB) Department of Obstetrics and Gynecology, Greenville Hospital System University Medical Center, Greenville, South Carolina
2	**COMPARISON OF TWO "ONE-STEP DISPOSABLE CHAMBERS" FOR SPERM COUNT AND MOTILITY ASSESSMENT** **Kelly S. Athayde, TS(ABB),** Anitra Patrick, Debra Garlak, Reda Mahfouz, Rakesh K. Sharma and Ashok Agarwal, PhD, HCLD(ABB) Andrology Laboratory and Reproductive Tissue Bank, Glickman Urological and Kidney Institute, Cleveland Clinic, Cleveland, Ohio
3	**EFFICACY OF DOUBLE DENSITY GRADIENT TECHNIQUE IN IMPROVING SPERM MORPHOLOGY IN SEMEN SAMPLES PREPARED FOR ASSISTED REPRODUCTION** **Kelly S. Athayde, TS(ABB),** Reda Mahfouz, Anitra Patrick, Debra Garlak, Rakesh K. Sharma and Ashok Agarwal, PhD, HCLD(ABB) Andrology Laboratory and Reproductive Tissue Bank, Glickman Urological and Kidney Institute, Cleveland Clinic, Cleveland, Ohio
4	**EVALUATION OF A BASOPHIL HISTAMINE RELEASE ASSAY** **Stephanie Fausett BS,** Mickie Kerr MT(ASCP), John F. Halsey PhD, HCLD(ABB) IBT Laboratories, Lenexa, Kansas

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5	**EVALUATION OF IN VITRO DIAGNOSTIC TESTS FOR DIAGNOSIS OF CHRONIC AUTOIMMUNE URTICARIA** **Mickie Kerr, MT(ASCP),** Michelle Altrich, PhD, and John F. Halsey, PhD, HCLD(ABB), IBT Laboratories, Lenexa, Kansas
6	**LEAN (98% Fat Free) ELECTRONIC ART MANAGEMENT SYSTEM** **K. Larose, MT(ASCP),** M. Mersol-Barg, MD, M. Parker, MT(ASCP), J. Spartz, MT(ASCP), M. Stahler, PhD, SMART Laboratories, Birmingham, Michigan
7	**PREGNANCY FOLLOWING THE TRANSFER OF A BLASTOCYST DEVELOPED FROM A 72 HOURS EMBRYO RETAINED ON THE CATHETER AFTER A DIFFICULT TRANSFER** **Roxana Napolitano, PhD,** and Alvaro Alosilla Fonttis, MD, Centro de Reproducción, La Plata, Buenos Aires, Argentina
8	**RELATIONSHIP BETWEEN DAY 3 EMBRYO MORPHOLOGY AND EUPLOIDY IN EGG DONATION CYCLES** **Catharine Adams, PhD, HCLDELD (ABB),** Linda Anderson, TS(ABB), Lynn Wheeler and Samuel Wood Reproductive Sciences Center, La Jolla, California
9	**SUPPLEMENTATION OF CULTURE MEDIA WITH ZWITTERIONIC BUFFERS SUPPORTS SPERM FUNCTION AND EMBRYO DEVELOPMENT WITHIN THE ELEVATED CO_2 LEVELS OF THE LABORATORY INCUBATOR** **Best Poster Presentation** **Jason E. Swain** and Thomas B. Pool, Ph.D., HCLD(ABB) Fertility Center of San Antonio, San Antonio, Texas

COMPARISON OF THE MANUAL, CASA, AND SCA METHODS FOR SEMEN ANALYSIS REPORTING

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Objective: To determine the differences in concentration and motility among three semen analysis methods: manual method (as described by WHO), CASA (Computer-Automated Semen Analyzer; Hamilton Thorne Research, Beverly, MA), and SCA (Sperm Class Analyzer; Microptic S.L., Barcelona, Spain).

Design: Prospective comparison study of using manual, CASA, and SCA methods of analyses.

Setting: A hospital-based andrology laboratory.

Patients: Male partners of couples undergoing infertility evaluation.

Materials and Methods: Fifty semen samples were collected and analyzed at the Greenville Hospital System University Medical Group andrology lab from December 6, 2007 to February 23, 2008. Using MicroCell^{TM} counting chambers (San Diego, CA), measurements of sperm concentration in the range of 5.5 to 250 x 10^6 were performed via manual, CASA, and SCA. These same chambers and methods were used for motility parameter comparisons.

Results: There were no significant differences among the manual, CASA, and SCA in regards to sperm concentration (P=0.9). While the same holds true for overall motility reporting with the manual, CASA, and SCA (P =0.9), there was significant differences between the three semen analysis methods in the motility parameter of forward progression at the rapid (4), medium (3), and slow (2) level (P = .0001 at each level). However, there was no significant difference between the three analysis methods at the forward progression static level, which classifies non-motile sperm (P = 0.4).

Conclusions: The manual, CASA, and SCA methods for reporting semen analysis results produced comparable sperm concentration regardless of different degrees of sperm concentrations. Overall motility values also were compared among the methods tested. However, there were significant differences in the motility aspects of the forward progression parameter. These differences in forward progression may be explained by the different operational variables associated with each individual method.

Key Words: CASA, MicroCell Chamber, SCA, Sperm, validation

COMPARISON OF TWO "ONE-STEP DISPOSABLE CHAMBERS" FOR SPERM COUNT AND MOTILITY ASSESSMENT

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Introduction: Many laboratories have adopted "one step" disposable chambers because of the ease in assessing sperm count and motility simultaneously. Disposable chambers also avoid slide opacity or contamination that may occur from a previous sample (as seen in Makler and Neubauer counting chambers) that could interfere with the accuracy of result. Our objective was to compare a new (CellVu®) counting device with an established (Microcell®) two "one-step disposable chambers" in our Andrology lab for manual sperm count and motility assessment**.**

Material and Method: Twenty-seven samples were evaluated in duplicate each chamber by the same observer. Nineteen samples from patients seeking fertility evaluation and eight donor samples were included. Five µl of well mixed semen was used for each assessment.

Results: The new chamber showed no defects upon sample loading independent of their viscosity status compared to about 10% of the Microcell chambers showing defects in sample loading. Semen quality was similar between the two chambers (Table 1).

Conclusion: We validated Cell Vu® chamber for routine use in our practice. It showed no problems with sample loading compared to Microcell slides. It is a good practice to validate the results of newly selected counting chamber as it can be used as part of the Quality Assurance program.

Table 1

	Microcel® chamber (n = 27)	**Cell Vu® chamber (n = 27)**	**P value**
Concentration ($x10^6$/ mL)			**0.99**
	60.3 (31.0, 142.5)	57.7 (31.0, 137.5)	
Motility (%)			
	72.0 (52.0, 84.0)	71.0 (52.0, 83.0)	**0.87**

Values are median (25^{th} and 75^{th} percentiles).
*P <0.05 was considered statistically significant using Wilcoxon Rank Test.

EFFICACY OF DOUBLE DENSITY GRADIENT TECHNIQUE IN IMPROVING SPERM MORPHOLOGY IN SEMEN SAMPLES PREPARED FOR ASSISTED REPRODUCTION

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Introduction: The goal of processing semen samples for ART is to improve pregnancy results by providing superior quality of sperm. We sought to evaluate the efficacy of double density gradient (DDG) technique in selecting good quality sperm for intra uterine insemination IUI.

Material and Method: In a prospective study, we evaluated 28 semen samples from patients selected for IUI. Semen samples were prepared by a two-layer density gradient (40%-80%) (Sage In-vitro Fertilization, Pasadena, CA). Smears were prepared to assess sperm morphology by WHO (3rd edition) and strict criteria in neat as well as post-wash semen specimens.

Results: Samples prepared by density gradient showed significantly higher percentage of normal sperm forms both by WHO ($p = 0.015$) and strict criteria ($p = 0.037$). Washed samples had a significantly smaller percentage of abnormal sperm morphology by strict criteria. Prepared semen showed absence of bicephalic head anomalies and a significant reduction in the percentage of tail abnormalities ($p = 0.003$) and round cells ($p = 0.001$).

Conclusion: Sperm preparation by double density gradient technique should be a method of choice for IUI patients. DDG improves the percentage of normal sperm forms as well as a significant reduction in abnormal sperm forms and round cells.

EVALUATION OF A BASOPHIL HISTAMINE RELEASE ASSAY

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Background: The activation and degranulation of mast cells and basophils are responsible for most of the symptoms of Type I allergic disease. For most allergens, the identification of the antigens that trigger the disease can usually be effectively determined by skin testing or in vitro allergen-specific IgE testing. However, adverse drug reactions (ADR) are very common and skin and in vitro tests to identify a causative agent for an ADR are not available for most therapeutic drugs. In addition, since most ADRs are not IgE-mediated, tests to sort out the mechanisms involved would be helpful in the assessment of these reactions.

Objectives: The purpose of this research was to develop and standardize a basophil histamine release test (BHRT) for use in the evaluation of drug allergen sensitivities.

Methods: Basophils were enriched from sodium heparinized whole blood and cultured with standardized, commercially prepared drugs as well as negative and positive controls. The stimulation buffer was used as the negative control and N-formyl-Met-Leu-Phe (fMLP) was used as the positive control. After brief cell stimulation, the cell culture supernatants were removed and the amount of histamine released from the basophils was measured by a competitive binding ELISA that uses a five point standard curve. Drug specific responses were determined by subtracting the concentration of histamine released in unstimulated cultures from the concentrations released following culture with the drug. Reference ranges were determined by calculating the mean plus two standard deviations for the control population.

Results: 1) The Histamine ELISA has a reproducibility of 9.5%. 2) The negative control for the normal donors varied from 1.6 to 5.5 ng/mL with a mean of 3.4 ng/mL and a standard deviation of 1.2. 3) The patient's negative control value was subtracted from each drug result and the difference was compared to the normal range for each drug based on twenty normal donors.

Conclusions: Normal reference ranges were determined for 27 drugs with the BHRT that was developed. These studies indicate that patients with values outside the reference range are sensitized and these patients should be further evaluated before therapeutic administration of the drug. Since placebo controlled challenge studies are difficult to perform, the true predictive value of the test is unknown for most drug allergens.

EVALUATION OF IN VITRO DIAGNOSTIC TESTS FOR DIAGNOSIS OF CHRONIC AUTOIMMUNE URTICARIA

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Background: Chronic Urticaria (CU) is estimated to affect 0.1% of the population. Although it is rarely fatal, it can have significant adverse effects on the quality of life for the individual with the disease. It is estimated that 30 – 40 % of patients with CU have an autoimmune component such as antibodies to FcεR, IgE or FcεRII/CD23. Diagnosis is often made in the physician's office using the Autologous Serum Skin Test (ASST), which is cumbersome to perform and very subjective to read.

Objective: Our purpose was to evaluate and compare two in vitro laboratory tests for the presence of the autoimmune component of CU as well as to evaluate the concordance of plasma versus serum as the sample matrix.

Methods: We performed two functional tests, histamine release and CD63 upregulation, to detect autoantibodies proposed to be relevant to autoimmue CU. Both sera and citrated plasma were evaluated in the histamine release assay. Patients from an allergy practice who fit established criteria for CU were selected. Samples were stored at -20ºC for up to 30 months. Initially, basophils from a single non-atopic donor were incubated with patient plasma, patient serum, buffer or anti-IgE as a positive control. Following ex-vivo incubation, the cells were analyzed for CD63 expression (a basophil activation marker) and the supernatants were recovered for histamine analysis by ELISA. A positive result in each test was defined as a value exceeding 2 standard deviations (SD) above the mean of a control population.

Results: Using the assigned cutoffs for a positive result, there was a high correlation between the CD63 upregulation and histamine release assays, but the histamine release assay was more sensitive. Sera from CU patients produced a higher mean histamine release, 23 percent, compared to citrated plasma, 12 percent.

Conclusions: These data show that basophil histamine release can be used as an in vitro method to identify the autoantibodies in CU patient's serum and plasma and that serum is the preferred specimen for use in this assay.

LEAN (98% FAT FREE) ELECTRONIC ART MANAGEMENT SYSTEM

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We have developed a user-friendly electronic relational database system that has plug and play convenience for new and established small and medium size ART Laboratories providing efficient management of patient and QC data. The design is driven by the patient's demand for a high level of quality assurance and successful outcome. The system is also very responsive to CLIA requirements for high complexity laboratories and FDA guidelines for management of HCT/Ps. SMART Labs recently received positive feedback from CAP and FDA inspectors which provided us with independent validation of the efficacy and advantages of our Lean Electronic Management System and encouraged us to present the software design and user demonstration in this forum. The system was designed by the laboratory staff rendering it practical and user-friendly for the entire ART team. The database architecture provides great flexibility and power to create reports, track trends and allows the system to evolve in response to changing demands in the laboratory or medical settings. The choice of software provides user flexibility to choose mixed computer operating system environments from which to work. The server-based system provides distribution of the electronic information throughout the center's widely distributed workstations allowing for immediate input and access of information. This results in efficient and effective analysis, decision-making and throughput in the delivery of highest quality patient care. The electronic management system (EMS) integrates ART laboratory operations with medical clinic operations. From the laboratory perspective, we maintain electronic andrology and IVF accession logs that track relevant QC (turn-around-times, performance metrics) and calculate test volumes and statistics. We also use quality control packs to track all lot numbers and their related QC testing. Utilizing the same database platform, gamete and embryo development is electronically managed from pretreatment testing to gamete collection, through transfer and cryopreservation. It allows management of FDA requirements for labeling, validation and tracking of donor gametes and embryos. From the medical practice perspective all aspects of the patients' clinical ART monitoring and treatment are integrated into the system database. Our Lean Electronic ART Management System may serve as a useful plug and play tool to those developing, starting or within established ART Centers. Its time and space saving features benefit the day-to-day clinical and laboratory operations and ultimately optimizes delivery of care to your patients.

PREGNANCY FOLLOWING THE TRANSFER OF A BLASTOCYST DEVELOPED FROM A 72 HOURS EMBRYO RETAINED ON THE CATHETER AFTER A DIFFICULT TRANSFER

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Objective: To report a successful pregnancy achieved after transferring a blastocyst developed from a 72 hours embryo, which had been retained on the catheter in a difficult transfer.

Design: Case report.

Patients: a couple undergoing in vitro fertilization (IVF).

Interventions: IVF followed by a difficult transfer. Three oocytes were obtained. P1 medium (Irvine Scientific, Santa Ana, CA) supplemented with 3 mg/ml of human serum albumin (Irvine Scientific, Santa Ana, CA) was used for culturing the oocytes and insemination, only one zygote resulted. P1 medium supplemented with 10% synthetic serum substitute (SSS, Irvine Scientific, Santa Ana, CA) was used to support development of the zygote to the eight cell stage, and later to the blastocyst stage. The embryo transfer (ET) was performed on the third day. In this case the ET was traumatic and bloody. The fact that the embryo had been retained on the catheter was noticed. Then, it was decided to leave the embryo in culture medium. The embryo was cultured to the blastocyst stage in a single medium, P1 supplemented with 10% SSS. A second transfer was performed.

Main outcome measures: development of a blastocyst and pregnancy.

Results: A 72 hours embryo, which had been retained on the catheter during a traumatic and bloody transfer, was cultured to the blastocyst stage. On day 5 the embryo was an early blastocyst with a clear inner cell mass (ICM), in the morning of day 6 it was an expanding blastocyst with a normal amount of ICM. Successful pregnancy was achieved after transferring this blastocyst.

Conclusions: In cases of difficult transfers, is reasonable to leave the embryos in culture medium, and wait for the blastocyst stage. This allows a second transfer to be performed without embryos and cervix under the effect of stress. Culture conditions, which support optimal development of the cleavage stage embryo do not support good blastocyst development and differentiation. In spite of these, the unique embryo we had was able to develop to the blastocyst stage in a standard medium. It has been hypothesized that the capacity of an embryo to develop into blastocyst stage depends primarily on the embryonic genome.

RELATIONSHIP BETWEEN DAY 3 EMBRYO MORPHOLOGY AND EUPLOIDY IN EGG DONATION CYCLES

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Introduction: Although egg donation results in high pregnancy rates, there is an increasing need for reducing the high multiple gestation rates by transferring fewer embryos. Preimplantation genetic diagnosis with aneuploidy screening (PGD-AS) may improve embryo selection in egg donor cases, which typically have high numbers of embryos, and as shown in recent studies, have higher than expected aneuploidy rates (~50%). The aim of this study was to determine which features of day-3 embryo morphology predict euploidy.

Materials and Methods: A retrospective review of 62 donor cases with PGD-AS performed in a private infertility clinic over a 2-year period. Multi-color FISH for chromosomes 13, 18, 21, X and Y was applied to single cells.

Results: A total of 562 embryos were analyzed (mean of 9.1/case). Embryos were graded at the time of biopsy (~67h post insemination) according to cell number, amount of cellular fragmentation (grade A: 0-10%, grade B: 10-20%, grade C: 20-30%), evidence of cellular compaction and thickness of the zona pellucida. The overall aneuploidy rate was 41%. There was no correlation between cell number and euploidy, although embryos with 8 cells had significantly higher euploidy rates (73%) than embryos with less than 8 cells (46%) and more than 8 cells (55%) ($p<0.001$). Cell fragmentation was highly correlated with aneuploidy. Grade A embryos had significantly higher euploidy rates compared to grade B embryos (71% vs. 49%, $p<0.0001$) and grade B embryos had significantly higher euploidy rates than grade C embryos (49% vs. 13%, $p<0.001$). There was no significant difference in euploidy rates between compacted (61%), slightly compacted (68%) and non-compacted embryos (58%). Embryos with normally thick zonas (15-20μm) had significantly higher euploidy rates than embryos with excessively thick zonas (≥25μm) (61% vs. 38%, $p<0.05$), but comparable to embryos with slightly thicker zonas (20-22 μm; 56%).

Conclusions: Cell fragmentation and abnormally thick zonas in day 3 cleavage stage embryos were predictive of aneuploidy. However, other morphological criteria, cell number and degree of compaction, did not correlate with euploidy. Even though many high grading embryos in these cases are chromosomally normal, there are also significant numbers of slow and fast dividing embryos that are euploid, for which PGD-AS seems to provide a valuable selection tool.

SUPPLEMENTATION OF CULTURE MEDIA WITH ZWITTERIONIC BUFFERS SUPPORTS SPERM FUNCTION AND EMBRYO DEVELOPMENT WITHIN THE ELEVATED CO_2 LEVELS OF THE LABORATORY INCUBATOR

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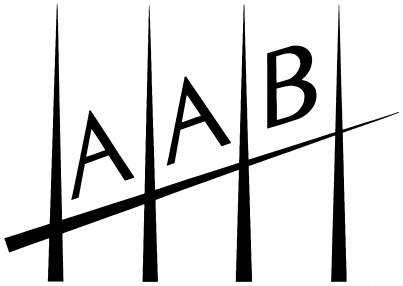
Maintenance of a stable media pH used for gamete and embryo culture in clinical IVF is crucial for proper functionality of various intracellular signaling pathways and biochemical processes, which can dramatically impact subsequent embryo development. Current culture media are pH buffered using $NaHCO_3$, which gives the desired pH within the elevated CO_2 environment of the incubator. However, $NaHCO_3$ is a weak buffer and can result in dramatic pH shifts outside the incubator. Therefore, improved buffering capacity of culture media, more resistant to perturbations in pH, may improve embryo quality. A series of synthesized organic buffers, known as Good's buffers, offer a means by which to improve buffering capacity of culture media. We demonstrate that addition of two Good's buffers, HEPES or MOPS, results in increased buffering capacity in response to an acid/base challenge compared to traditional $NaHCO_3$ buffered culture media. To begin to determine applicability of media supplemented with synthetic organic buffers for use within the laboratory incubator in clinical IVF, we examined their effects on sperm function and embryo development. Sperm from normospermic men were isolated by density gradient column and initial counts taken. Utilizing P1 + 0.3% HSA as a base/control medium, media were supplemented with either 25mM HEPES, 25mM MOPS, 50mM HEPES or 50 mM MOPS. All media contained 25mM $NaHCO_3$, maintained a pH of 7.25-7.30 when cultured within the same incubator (approximately 5% CO_2), and had an osmolarity between 280-290 through adjustment of NaCl levels. Sperm samples were distributed evenly between five treatments: 1) Control 2) 25mM MOPS 3) 50mM MOPS 4) 25mM HEPES and 5) 50mM HEPES and placed overnight into the incubator (approximately 5% CO_2). Initial studies (n=3) showed similar motility and forward progression following 24h incubation between control $NaHCO_3$ buffered media versus media supplemented with either 25 or 50mM MOPS or HEPES. To further examine effects of synthetic buffers on sperm function, we examined ability of human sperm to undergo decondensation following microinjection into hamster oocytes and culture for 4h in a P1-based medium supplemented with either 50mM MOPS or HEPES. Comparable decondensation rates were obtained in all media (con: 60% n=15, MOPS: 62% n= 13, HEPES: 67% n=15). Additional experiments have validated use of these double-buffered media to support mouse blastocyst development. These studies lay the groundwork for utilization of culture media with enhanced buffering capacity in clinical IVF.



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