

Abstracts Presented in 2011

Oral Presentations

Friday, May 13, 2011 • 3:15 p.m.-5:15 p.m.

A Novel Sperm Morphology Algorithm That Improves Repeatability And Stability While Reducing Overly Critical Analysis

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Problem: Ample data show that most laboratories using Strict sperm morphology become overly critical in their analysis until very few normal sperm are reported. In many centers, morphology has lost its predictive value for assisted reproductive therapies. Recent publication of WHO reference ranges for sperm morphology encompass median and upper limits that many laboratories never attain. Intra-observer and inter-laboratory variations are excessively large as revealed in competency and proficiency testing, suggesting that many different interpretations of the Strict method are in use. Analysis is subject to shift and drift caused by many influences and stability of measurement is difficult to maintain.

Method: We reviewed photographs and definitions of normal, borderline, and abnormal sperm from available atlases, published papers and the WHO 5th semen analysis manual, along with data from an NIH grant surveying classification of 155 sperm by 99 international experts. We created a classification algorithm based on a standardized, logical evaluation order and rational, unambiguous cell descriptions. The method relies on separate identification and defined classification of borderline normal forms as described by Menkveld in 1990.

Results: 143 archived morphology smears were analyzed with the algorithm and compared to original values. Subsequently the algorithm was used to analyze 436 archived smears. The distribution of values was comparable to the WHO 5th reference ranges with less than 10% of the values in the 5th centile (<4% normal Strict morphology) and a median of 17% normal. Regression analysis of 170 samples showed excellent inter-observer correlation with a correlation coefficient of 0.88. The

stability of the method was excellent over 8 months of analysis with a slope of 0 for each of two analysts. Using the algorithm reduced analysis time by approximately half for a full teratozoospermia differential from 30 to 15 minutes.

Conclusions: Use of the morphology algorithm provided results that were repeatable and stable, with medians and distributions similar to WHO 5th reference ranges. Because borderline sperm are classified independently, the algorithm can be used to obtain percent normal forms for either traditional or Strict morphology schemes in the same analysis.

Anti-Mullerian Hormone As A Predictor Of Live Birth Rates

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Objective: AMH (anti-mullerian hormone) performs various physiological functions. AMH is routinely used clinically to help determine ovarian reserve status and subsequently gonadotropin stimulation protocol. Data are lacking that relate AMH levels to live birth outcome. The objective of this study was to determine if AMH levels can successfully predict live birth outcomes of the corresponding IVF (in-vitro fertilization) cycle.

Design: Retrospective data analysis of patients undergoing IVF at a private fertility clinic.

Materials and Methods: Patients diagnosed with polycystic ovarian syndrome, diminished ovarian reserve, and donors were excluded from the study. Patients were divided by age, <35 (group 1), 35-37 (group 2), >37 (group 3), to determine if AMH was a valid predictor for live births. A receiver operator curve, and ANOVA were used where appropriate and P was set at <0.05.

Traditional PRE	Traditional POST	Strict PRE	Strict POST	WHO 5 th TTP <12 Months	WHO 5 th Unscreened
21%	30%	4%	18%	15%	14%

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Results: A total of 98 patients were included in this study. AMH levels between groups were significant, 2.5 ± 1.4 , 1.70 ± 0.9 , and 1.5 ± 1.6 ng/mL for groups 1, 2, and 3, respectively ($P=0.0133$). For group 1 (30.4 ± 2.5 years), 44 of 68 (64.7%) patients gave birth (criterion >2.77 , specificity 87.5, sensitivity 50.0, $P=0.0084$). For group 2 (35.6 ± 0.7 years) 10 of 17 (58.8%) patients gave birth (criterion >1.36 , specificity 85.7, sensitivity 70.0, $P=0.0589$). Of the 13 patients (39.1 ± 1.3 years) in group 3, 5 (38.5%) gave birth (criterion >0.9 , specificity 50.0, sensitivity 100.0, $P=0.4074$).

Conclusions: AMH has been shown to be a valid predictor of ovarian reserve and response to gonadotropin stimulation. This is the first data of our knowledge that follows AMH values out to live birth rates, as opposed to chemical and clinical pregnancy. Our data show that for patients under 35 years AMH is strong predictor for live birth. As the patient ages the trend becomes less significant.

Comparison Of The Effectiveness Of Programmable Slow-Freezing (SF) Vs. Vitrification (VT) For Cryopreservation Of Human Eggs

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Objective: To compare post-thaw survival, fertilization and cleavage rates, and the percent of transferable embryos in relation to subsequent implantation and pregnancy for human oocytes cryopreserved using either SF or VT.

Design: A retrospective analysis.

Materials and Methods: Two different techniques of cryopreservation, SF and VT (Cryotop), were used during the overlapping time periods. In some patients with more than 10 eggs, both cryopreservation techniques were used. A total of 66 cryopreserved oocytes were thawed and fertilized with ICSI; 41 of these were cryopreserved by SF and 25 by VT. The resulting embryos were transferred during eight transfer cycles (ET).

Results: As demonstrated in Table 1, oocyte survival and fertilization rates were comparable for both techniques. The cleavage rate and the percentage of transferable embryos were significantly higher after VT. In three

patients whose oocytes were cryopreserved using both techniques, only VT eggs generated transferable embryos. All three ET cycles following egg cryopreservation with SF resulted in pregnancies and live birth (one singleton and two sets of twins) with an implantation rate of 62.5%. Of five ET cycles using previously vitrified oocytes, three resulted in pregnancy (one ongoing and two delivered - one singleton and one set of twins) with an implantation rate of 40.0%.

Table 1 Comparison Between SF and VT

	Slow-freezing	Vitrification	P Value
# Eggs Thawed	41	25	
# Survived (%)	40 (97.6%)	25 (100%)	>0.05
# Fertilized (%)	30 (75%)	3 (90.9%)	>0.05
# Cleaved (%)	20 (66.7%)	21 (95.0%)	0.048
# Transferable Embryos (% per Thawed Egg)	8 (19.5%)	12 (48%)	0.025

Conclusions: Oocyte VT results in a higher survival, fertilization, and cleavage rates and provides a larger percentage of transferable embryos than the slow-freezing methods. However, if transferable embryos are obtained and transferred, implantation and pregnancy rates are similar for both techniques.

Dipeptide Glycine Acts As An Osmolyte During Mouse Preimplantation Embryo Development *In Vitro* And Supports Blastocyst Formation In High Osmolality Media

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Introduction: Amino acids are important components of embryo culture media, serving as metabolic substrates and homeostatic regulators. Of particular interest is the amino acid glycine. Glycine is beneficial for embryo development and is a potent osmolyte. A potential improvement may lie in the use of dipeptide forms of amino acids. For embryo culture, dipeptides of glycyl-glutamine and alanyl-glutamine are superior to glutamine, likely due to lower ammonia production. However, information on the use of other dipeptides or their ability to act as osmolytes in embryo culture is lacking. Therefore, the objective of this study was to examine the impact of dipeptide forms of glycine on mouse embryo development *in vitro*.

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Materials and Methods: Media for culture experiments consisted of Human Tubal Fluid medium (HTF) + 5% SSS protein. Embryos were cultured in groups of 10/500µl media covered with 300µl oil in 4-well dishes for 96h in ~6% CO₂. pH of all media were kept between 7.27-7.32. Osmolality of control media was ~285mOsm, while hyperosmotic media (~320mOsm) was formulated by adjusting NaCl levels. Ability of dipeptide glycine forms to act as osmolytes in high osmolality media and support embryo development in comparison to individual amino acids was examined. Positive controls consisted of 285mOsm media with no amino acids, while negative controls consisted of 320mOsm media with no amino acid. Treatments included 320mOsm media supplemented with 1mM alanyl-glycine, 1mM glycine-alanine, 1mM glycine, or 1mM alanine. Additionally, treatments of 2mM glycine or 1mM alanine + 1mM glycine were tested to account for differences in concentrations as dipeptides are cleaved. Finally, ammonia production of dipeptides and individual amino acids was examined using a commercially available colorimetric OxiRed based assay. Data were collected over 3-4 replicates and statistical differences determined using Tukey multiple comparison test.

Results: Data indicate that dipeptide glycine can serve as an osmolyte and support mouse blastocyst formation in media with high osmolality. Both 1mM alanyl-glycine (75%) and 1mM glycyl-alanine (80%) yielded similar blastocyst development as 1mM glycine (85%) and 1mM alanine (70%). Additionally, both 2mM glycine (80%) and 1mM glycine + 1mM alanine (85%) yielded similar rates of blastocyst formation as dipeptides or 1mM amino acids. All treatments yielded similar results to 285mOsm controls (92.5%) and were

significantly higher than 320mOsm negative controls (37.5%). Similar results were obtained for rates of expanded blastocysts and hatching blastocysts. Interestingly, though not statistically significant, developmental rates for glycine and alanine + glycine were consistently higher than alanine or either dipeptide form. Dipeptide glycine forms produced significantly less ammonia than glycine alone, though overall amounts were minute.

Conclusions: Dipeptide glycine forms can serve as osmolytes for mouse preimplantation embryos and permit blastocyst formation in high osmolality media. However, use of individual amino acids appear to be more effective, raising the question of how embryos transport/utilize dipeptide amino acid. Additionally, dipeptide glycine produces less ammonia than glycine, though overall amounts for all treatments were small. Therefore, there does not seem to be a dramatic benefit of using dipeptide glycine as an osmoregulator and the efficacy of other commonly used dipeptide amino acids to act as osmolytes, such as alanyl-glutamine and glycyl-glutamine, should be examined.

Dynamics Of Nitric Oxide, Altered Follicular Microenvironment And Oocyte Quality In Subjects With Endometriosis

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Objective: To study components of follicular microenvironment with respect to free radical dynamics, oocyte quality and clinical ART

outcomes among subjects with (group A, n=8) and without endometriosis (group B, n=20).

Design: Prospective cohort study.

Setting: University-based ART center.

Patients: Subjects with and without endometriosis undergoing ART.

Intervention(s): Follicular fluid, granulosa cells and immature oocytes as well as clinical demographics, ART data and outcomes were studied and compared.

Main Outcome Measures: Nitrate/nitrite ratio and iron metabolites in the follicular fluid; peroxynitrite formation and apoptosis in the granulosa cells; status of cortical granules, microtubule dynamics, spindle morphology and zona pellucida dissolution time for in vitro matured oocytes as well as outcomes of the ART cycle in terms of the fertilization, embryo quality, implantation and live-birth rates.

Result(s): ART data regarding patient demographics, ovarian stimulation characteristics as well as oocyte and embryo numbers and implantation rates were similar between groups A and B. Similarly, the pregnancy rates were similar between groups A (40.0%) and B (61.1%) respectively. Follicular fluid nitrate/nitrite ratios were significantly higher, while the fraction of immature oocytes matured in vitro was significantly lower in group A compared to group B (33.7 versus 67.6%, $P=0.044$). Incidence of apoptosis and positive peroxynitrite staining in the granulosa cells and abnormalities among in vitro matured oocytes (namely, increased zona pellucida dissolution timings, cortical granule loss, ooplasmic microtubule dynamics and spindle disruption) were significantly higher in patients from

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group A that did not conceive during the ART treatment cycle compared to other subjects in groups A and B irrespective of their ART outcomes ($P < 0.005$). Iron studies revealed significantly higher follicular fluid transferrin levels in older (>35 year old) subjects ($P = 0.022$) and among subjects that did not conceive, irrespective of their endometriosis status ($P = 0.04$).

Conclusion(s): Increased follicular cellular apoptosis, oocyte abnormalities and increased nitrate and peroxynitrite indicate that endometriosis adversely impacts follicular microenvironment and oocyte quality in endometriosis. Altered Fe metabolism could be a common mechanism for both, age- and endometriosis-related infertility.

Key Words: Endometriosis, oocyte aging, oocyte quality, nitric oxide, superoxide, peroxynitrite

IVF Clinical Pregnancy Rate Is Associated With Embryo Quality And Number Transferred On Day 3 But Not With Pronuclear Score On Day 1

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Objective: The association between pronuclear (PN) scoring and clinical pregnancy success following IVF

is controversial. Our study objective was to further investigate PN scoring and clinical pregnancy rate in a large sample size.

Materials and Methods: Patients (19-46 years) undergoing IVF/ICSI cycles at Montefiore's Institute for Reproductive Medicine and Health between January 2006 and December 2009 were included in our study. We analyzed fresh day 3 cycles only with autologous oocytes and partner's fresh sperm (n=344). A total of 1899 embryos were included. We compared pregnancy rates from non-PN scored embryos (Group 1, n=835) to PN scored embryos (Group 2, n=1064). PN scores (Scott et al., 2007) were assessed 16-18 hours after insemination. We hypothesized that embryos from Group 2 would result in better pregnancy rates than Group 1 because embryo selection on day 3 was refined by PN scoring on day 1. We developed composite scores by patient based on embryo disposition; each patient had a composite embryo score for embryos transferred, frozen and discarded. We also assessed traditional embryo grading derived from cell number, fragmentation and cell symmetry. Both groups had day 3 embryo grading performed. Additional variables considered included patient age, number of mature oocytes retrieved and number of embryos available on day 3. Data analysis included chi square and t test to determine if PN scoring improved clinical pregnancy rate, and to compare the additional variables. P values < 0.05 were considered to be statistically significant.

Results: Clinical pregnancy rate between Group 1 and Group 2 were not significantly different ($p = 0.91$). Patient data for Group 2 is presented in Table 1. Clinical pregnancy rate was significantly associated with female age, number of mature oocytes retrieved, number of day 3 embryos and mean grade of embryos transferred on day 3 (p values < 0.05).

	Clinical Pregnancy n=128	Not Pregnant n=216	P-value
Female age, years	34.5 (4.3)	36.1 (4.5)	<0.01
Max FSH (mIU/mL)	7.6 (2.7)	7.7 (2.3)	0.71
# Mature oocytes retrieved	10.0 (4.8)	8.6 (5.0)	0.04
# Embryos transferred	2.8 (0.8)	2.7 (1.0)	0.26
# Embryos on day 3	6.2 (3.4)	5.1 (3.3)	<0.01
Mean grade embryos transferred	1.6 (0.4)	1.7 (0.4)	<0.01
Mean PN score transferred embryos, n=948	2.1 (0.7)	2.0 (0.6)	0.63

Table 1: Data presented as mean (SD) or prevalence. Mean PN score includes only embryos transferred; 951 embryos of the original 1899 were either cultured out to day 5 or discarded.

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Conclusion: Previous studies have reported PN pattern data improving embryo selection and ultimately clinical pregnancy. PN scoring was not shown to improve clinical pregnancy rates in day 3 embryo transfers in our study. Mean grade of embryos transferred on day 3 continues to be a well-established, independent predictor of clinical pregnancy rate. This suggests that further refinement of embryo grading by PN scoring is not beneficial.

References: Scott, L., Finn, A., O’Leary, T., McLellan, S. and Hill, J. (2007) Morphologic parameters of early cleavage-stage embryos that correlate with fetal development and delivery: prospective and applied data for increased pregnancy rates. *Hum Reprod*, **22**, 230-240.

Improved In Vitro Blastocyst Production Using A Single Culture Condition For Combined Oocyte Maturation, Fertilization And Embryo Development: Lessons From The Domestic Cat Model

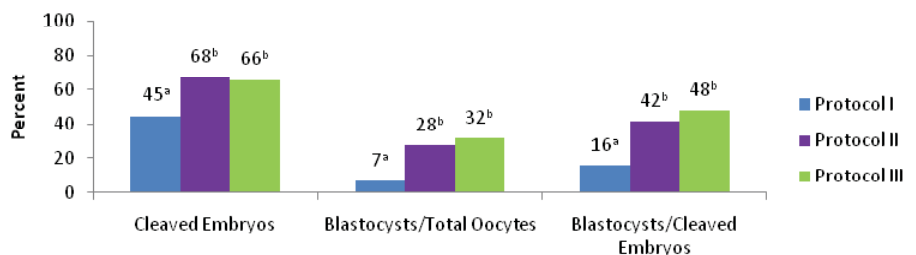
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Over the past 2 decades, multiple protocols have been developed to generate cat embryos in vitro, including our routine method using Eagle’s Minimal Essential Medium (Eagle’s MEM) for in vitro maturation (IVM) and Modified Ham’s F-10 Basal Medium (Ham’s) for in vitro fertilization (IVF) and embryo culture to the blastocyst stage. These conditions consistently yield 80% metaphase II oocytes after IVM, 50% cleaved embryos after IVF and 20% blastocysts (per cleaved embryo) after 7 days of culture. Using the domestic cat as an experimental model, our objective was to determine if a single medium could be effective for provoking ‘development’ from advancing maturation of intrafollicular, ovarian oocytes through fertilization and blastocyst formation. Immature cat oocytes (n = 361; 3 replicates) were collected from adult cat ovaries and randomly allocated to one of three treatments. In Protocol I, IVM was conducted (24-28 hr) in Earle’s MEM supplemented with 1 µg/mL FSH, 1 µg/mL LH, 1 µg/mL estradiol and 4 mg/mL bovine serum albumin, and then IVF with thawed cat spermatozoa and embryo culture in Ham’s supplemented with 5% (v/v) fetal calf serum (8 days; standard approach). In Protocol II,

a commercially-available blastocyst medium (no protein supplementation required) from Company A was used for IVM (supplemented with FSH, LH, and estradiol as above), IVF and embryo culture. In Protocol III, a commercially-available blastocyst medium from Company B was used for IVM (supplemented with FSH, LH and estradiol, as above, along with 4 mg/mL bovine serum albumin), and supplemented with 5% (v/v) fetal calf serum for IVF and embryo culture. Presumptive embryos were cultured for 8 days, fixed and stained with Hoechst and propidium iodide to assess stage of development. The percentages of cleaved embryos and blastocysts (relative to the total number of oocytes or relative to the total number of cleaved embryos) were significantly higher in Protocols II and III compared to Protocol I (Fig. 1). Results indicated that it is possible to increase the percentage of cleaved embryos by 1.5 times and at least triple the percentage of blastocysts in the cat using an alternative culture medium. While the precise cause for the striking improvement is unclear, it is notable that a single medium environment can meet the complex needs required for combined nuclear maturation, fertilization and advanced preimplantation embryo development.

Figure 1. Proportions of cleaved embryos and blastocysts after IVM, IVF and embryo culture in three different culture conditions.

^{a,b} Within the same metric, values with different superscripts differ ($P < 0.05$; Chi-square test)



The Learning Curve Associated With Mastery Of The Rapid I

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Objective: There has been an explosion in vitrification (vit) media and devices. Labs must become proficient with several of these devices in order to accept samples from other programs. The purpose of this study was to

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monitor training and mastery of a new device and media (Rapid I and Vitrolife media [Media B]) compared to our standard method [CryoTips and Irvine media (Media A)] using mouse embryos before beginning to use this device in the clinic.

Design: Prospective randomized design.

Materials and Methods: Experiment 1:

Initially 160 one-cell mouse embryos were thawed and cultured to blastocyst in Global media + 10% SSS. 40 embryos were vitrified in each of 4 groups: media A in CryoTip or Rapid I, and media B in CryoTip or Rapid I. A number of technical issues were identified. These issues were discussed with the manufacturer and modified.

Experiment 2: The experiment was repeated using the improved technique. 360 mouse embryos were thawed and cultured in Global media + 10% SSS. 80 multicell and 80 blastocysts were vitrified using media A with CryoTip and Rapid I; 80 multicell and 80 blastocyst embryos were vitrified using media B with CryoTip and Rapid I. All blastocysts had laser Assisted Shrinkage (AS) prior to vit. The remaining embryos were kept in culture for 24 hr as a control. After storage in LN₂ for 24 hr, multicell embryos were warmed and cultured to blast. Embryos vitrified as blasts were warmed after 24 hr in LN₂ and cultured overnight. A blast was identified as surviving if it had expanded and/or hatched 24 hr after warming.

Results: Experiment 1: Embryos vitrified in media A and CryoTips had significantly better survival than embryos vitrified in media A with Rapid I (100% vs 61%; P<0.05). Embryos vitrified in media B and CryoTips also had better survival than

embryos vitrified in media B with the Rapid I (97% vs 77%).

Experiment 2: After modifying our technique with the Rapid I, embryo survival after vit at multicell with the Rapid I improved (90% and 89% for media A and B). Embryos frozen at multicell in Rapid I formed similar numbers of Day 5 blastocysts with media A and B (92% and 91%) compared to CryoTips with media A and B (95% and 97%) and both were similar to fresh controls (95%). Furthermore, by Day 6 hatching rates from embryos frozen at multicell following vit in Rapid I with media B (90%) were better compared to Rapid I with media A (75%, P<0.05) and CryoTip with media A or B (79% and 74%).

Conclusion: Two closed-system vitrification devices, the CryoTip, and the Rapid I, were compared using DMSO-based media (Manufacturer A; Irvine Scientific) and PROH-based media (Manufacturer B; Vitrolife) to monitor successful mastery of the device and the methodology. Results showed that interchanging media and devices did not affect the survival or developmental potential of the mouse embryos once the technical aspects of the new device were mastered. Technical details unique to the Rapid I compared to the CryoTip included the necessity of using large volumes of media warmed to 37°C, using very small volumes of media to load the Rapid I in order to ensure that embryos were placed inside the aperture, adjusting the timing involved in placing the holding straw into the Smart Box and removal of the rod to prevent condensation and “popping” of the Rapid I inside the straw, control of the heat sealer, and timing of loading. Some differences exist between manufacturers of different types of vit media and devices; however these differences may be

overcome with proper training of technicians.

Supported by: Media from A was provided free of charge.

Poster Presentations
Friday, May 13, 2011
6:15 p.m.-7:30 p.m.

Assessment of Quality Of Expired Andrology Stain Used For Human Sperm Morphology Evaluation

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Objective: Andrology laboratories, in fertility clinics across the globe, heavily rely on the utilization of commercially available stains for differential diagnostic evaluation of human sperm morphology. The manufacturers specify an expiration date thus indirectly forcing the end users to discard the stain if not utilized by the set time. In this study the suitability of such stain when expired is investigated.

Study design: The quality of expired stain was evaluated and compared with that of unexpired control.

Materials and Methods: STAT III andrology stain (Origio, New Jersey) was evaluated at expired and unexpired conditions. Stain quality at expiration of 515 days was compared with that of an unexpired one (lot 7219 vs lot 0033) of the same stain kit (Kit 85316). At the time of evaluation, the expired and unexpired lots had been in the laboratory for 1060 days and 60 days, respectively. Both stain lots were properly handled and stored to protect them from dehydration and unneces-

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sary exposure to light. Precipitation and effectiveness of each stain component namely fixative, solution I (eosin) and solution II (azure) of both lots were assessed qualitatively and quantitatively (as appropriate) employing eight independent parameters. Comparison was based on quadruplicate replicates of the experiment using slides prepared with a normal semen sample. All slides (n = 24) were stained at one time following same staining technique as per manufacturer provided protocol. A trained experienced andrologist analyzed the slides in a blinded way.

Results: A significantly higher precipitation was noticed in all three components of the STAT III stain kit (fixative, solution I and solution II) in expired lot compared to unexpired lot. The increase in precipitation was in the following order: solution II > solution I > fixative. Slides stained with expired and unexpired stain exhibited same degree of clarity and brightness. Further, slides stained with expired stain yielded identical morphological scores compared to slides stained with unexpired stain (11.3±2.0% vs 11.8±2.1% at Kruger strict criteria). Head and tail size values (in ocular micrometer unit) were also identical in slides stained either with expired or unexpired stain (H: 4.7± 0.1 vs 4.6± 0.22; T: 49±2 vs 50 ± 1). No difference was seen in preserving sub cellular organelles in sperm head and neck in slides processed by expired and unexpired stains.

Conclusion: Identical morphology scores obtained from slides stained with unexpired and expired STAT III stain suggests that either i) aging effect on stain is marginal if appropriately handled and stored during use in laboratory or ii) sperm morphology is not an effective system in detecting aging related changes in stain quality if any.

Support: University Fertility Center, the University of Texas Medical Branch, Galveston, Texas.

Comparison Of Antimullerian Hormone, Day 3 Follicle Stimulating Hormone, And Antral Follicle Count As Predictors Of Live Birth Rates

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Objective: There are a variety of tests that clinicians use to gauge ovarian response prior to IVF (in-vitro fertilization). Previously, antral follicle count (AFC) and day 3 FSH (follicle stimulating hormone) were most often used to help predetermine ovarian response. Recently, AMH (anti-mullerian hormone) has been touted as being a better predictor of ovarian response when compared to AFC and FSH. The goal of this study was to determine which test, whether AFC, FSH, or AMH is a better predictor of IVF live birth rates.

Design: Retrospective data analysis of patients undergoing IVF at a private fertility clinic.

Materials and Methods: Patients had their AFC measured via ultrasound and blood drawn on day 3 for both FSH and AMH. A receiver operator curve was used where appropriate and P was set at <0.05.

Results: A total of 175 patients (31.9 ±4.9 years) were included in this study, 97 of 175 (55.4%) gave birth. The average AMH was 2.5 ±1.9 ng/mL (sensitivity 76.3, specificity 55.1, criterion >1.4, P=0.0016). The average AFC was 19.0 ±9.4 follicles (sensitivity 91.7, specificity 41.0, criterion >11, P=0.0001). The average day 3 FSH was 7.1 ±2.8 ng/mL (sensitivity 28.9, specificity 79.5, criterion >8.1, P=0.9068).

Conclusions: AMH and AFC provide a significant predictor for live birth rates compared to day 3 FSH values. AMH is extremely beneficial due to its ability to be measured at any time during the cycle, unlike AFC and FSH which require a measurement on a specific day. Furthermore, AMH doesn't require the use of a specialized technician or ultrasound machine.

**Equipment and Process Validation:
Hassle or Helpful**

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Introduction: In the ever changing arena of reproductive science, new methodologies, technologies, media, and equipment are constantly evolving and being implemented into infertility laboratories around the world. Validation of new equipment or processes is critical to safe and successful implementation. A prospective outline and subsequent

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data collection summary provides assurance that expected outcomes will be achieved.

Material and Methods: A validation plan should be written to include an outline of why and how the validation is being done. What is the purpose of the specific validation? Is it to determine if one process is comparable to another or is equipment brand X comparable to brand Y, or does it verify the design of a new process? The document should define the scope of the validation and state the expected outcomes. A summary of the system should be described and include the current and proposed changes that will affect the laboratory. It should also include the main points that will be validated, how it will be conducted with prospective, concurrent and retrospective review. State who is involved in the validation process and what are their specific responsibilities. Describe what standard operating procedures (SOP's), equipment, maintenance or samples will be needed in a step-by-step fashion. Develop data collection forms and prepare for analysis of the collected data; with acceptance criteria and expected outcomes.

When the data have been collected, summarize the process to ensure the original plan was followed. Note any deviations with clear explanation that may need to be incorporated into any final working documentation. Place the data into a summary format that can be reviewed with signature approval from designated, authorized personnel and, finally, create a file for validation documents that can be referred to in the future.

Conclusion: The planning and completion of equipment or process validation can be stressful and easily by-passed in a lab short on staff and long on case loads. Set aside time

to develop templates that can easily be completed by laboratory staff for the validation process. With proper planning and documentation it can aide in the overall success of patient outcomes and laboratory compliance.

How Do You Use Your Benchtop Incubator? An Embryomail Survey

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Introduction: During the past 10 years, a new generation of incubators has arrived on the IVF scene and now there are several manufacturers. With these new additions, and with their increasing popularity, embryologists now not only have a choice of brand of unit, but also have many other important variables to consider when deciding to switch to this style of incubator. As a measure of current state of practice, we posted a survey on Embryomail to assess how most labs are using these incubators.

Materials and Methods: The survey was developed on the site SurveyMonkey.com and was posted to EmbryoMail. The survey consisted of 23 questions and was divided into 5 categories: Brand of Incubator, Insemination, Post – Fertilization, Embryo Transfer and Quality Control.

Results: There were 87 respondents to the survey. The most common incubator cited was the Cook MINC incubator (70%) followed by the Planer BT-37 (22%). The two most common culture systems for standard insemination (IVF) were

multi-well dish (42%), microdrop (38%). Following ICSI, 86% culture in microdrops, with nearly two-thirds (63%) culturing oocytes in groups. The 60mm culture dish was most commonly used for embryo culture (42%) with 90% of respondents' culturing with the dish lid in place. Most respondents (74%) move embryos from their original culture dish into a separate dish prior to the transfer. The dish most used for transfer is the organ culture dish (45%) containing 1.0mL of media (70%) and no oil overlay (70%). Nearly all respondents used the same gas mixture in all bench top incubators in their labs (97%). During insemination (IVF), the preferred gas mixture was 6% CO₂ and 5% O₂. Most users set pH for insemination and embryo culture at 7.25-7.30 (66%). Almost half of respondents, (48%) test for pH using a meter, with 16% performing a color check or use pH paper, while 36% do no pH testing. Equilibration time was 13 hours to overnight for most respondents (58%). Temperature verification was performed by 79% of respondents by using a certified thermometer either as a surface check or within a vessel containing media or water. When asked about pregnancy outcomes, 76% of respondents have seen an increase in pregnancy rates, while 24% saw no change. No respondents reported a lower pregnancy rate with the use of the benchtop incubator.

Conclusion: These results provide a general survey of current practices of use of benchtop incubators. The benefits of benchtop incubators include reduced cost, space and, as evidenced by the majority of respondents to this survey, improved clinical outcomes.

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Improvement of Pregnancy Rate in Immunologically Infertile Patients Undergoing Prednisolone and IVF Program

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Sperm bound antibodies interfere with sperm function and post-fertilization events. Cervical antisperm antibodies bind to the sperm cause sperm immobilization. Seminal plasma antisperm antibodies impair sperm motility as well as viability.

The objective of the present work was to study the outcome of IVF and pregnancy rates in immunological infertile patients following prednisolone and IVF treatments. Micro agglutination test was used to determine positive antisperm antibodies in infertile men. The application of hamster sperm penetration assay (HSPA) in positive men with antisperm antibodies resulted in 48.7% (96/197) positive and 51.3% negative HSPA scores.

The positive HSPA patients were divided into control and treated groups. The treated group received 5 mg prednisolone for three months. The women had normal ovulatory cycles and reproductive and thyroid hormone concentrations. All patients were involved in IVF Program. The Beta-HCG and progesterone concentrations were assayed 12 to 14 days following embryo transfer to check pregnancy rate. The embryo implantation rate was examined by ultrasound five weeks after embryo transfer.

The sperm motility index and normal sperm morphology were significantly higher ($P < 0.01$) in the treated group compared to control group. The in vitro fertilization rate was significantly higher ($P < 0.05$) in the treated group compared to control group (70% vs. 60%). The pregnancy rate was significantly higher in the treated group than the control group (40% vs. 27%).

In conclusion, the application of prednisolone and IVF in immunological infertile men significantly improves in vitro fertilization and pregnancy rates.

Sperm Selection Technique During ICSI Yields Different Gender Ratio Among Embryologists

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Introduction: By natural pregnancy, the universal gender ratio is about 105 male to 100 female. By ICSI, it is the embryologist who selects a spermatozoon to fertilize an oocyte. Is the selection of spermatozoon really by random or a specific preference for certain appearance among embryologists? "Is there a difference in embryo gender ratio among embryologists?" is the question.

Materials and Methods: All pre-implantation genetic diagnosis (PGD) cases for aneuploidy are included in the study during 2007 to 2009. Totally 259 cases with 2070 embryos are included. The PGD examined chromosomes 13, 15, 16, 18, 21, 22, X, and Y. To avoid any additional sperm interference, all PGD cases were inseminated by ICSI. The definition for normal male is with XY sex chromosomes and XX sex chromosomes for female. The broad definition for male is the presence of Y chromosome and presence of at least 2 X chromosomes for female. One way ANOVA or Chi-square test used for the analysis of difference. Total data from 4 embryologists are included.

Results: Totally the gender ratio is 85 male: 100 female. It is significantly different ($p < 0.01$) from the universal gender ratio of 105:100. For 2007, the ratio was 87:100. This discrepancy was discussed during 2008. For 2009, the gender ratio became 96:100. The gender ratio of 2007 vs. 2009 was not significantly different but there was a change in trend. There was a significant difference ($p < 0.01$) between 2 embryologists. One embryologist significantly gave more male while the other embryologist significantly gave more female. The results showed there is a difference in selecting spermatozoa for ICSI.

Discussion: The spermatozoa selected for ICSI were following Strict Morphology by Kruger definition. It is possible that the interpretation of these criteria has slight variation. As the significant difference of embryo gender ratio among embryologists, the slight variation results in selection of different subpopulation of spermatozoa for ICSI. We are engaging to materialize the "slight variation."

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State Of The ART: What Are Current Laboratory Practices?

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A survey of laboratory directors listed in SART 2009 ART programs was conducted to evaluate current laboratory processes. Of the 392 ART programs listed in SART, e mail addresses were available for 92.8%. The remaining programs (7.2%) had no director listed and/or no e-mail available. Directors were e mailed directly and asked to follow a link to the 50 survey questions regarding general laboratory practices. The questions covered a range of items from philosophy of laboratory-patient contact; environmental standards; quality control; quality assurance, as well as some general procedures. The aim was to determine what commonalities exist between ART programs. Directors were asked to complete the survey for each laboratory directed as practice patterns may vary between locations.

100% of respondents reported currently being a laboratory director, with 72% of these being the director of a single laboratory; 28% directing 2 or more laboratories. Approximately one quarter of laboratory directors reported spending less than 25% or greater than 75% of their time performing hands-on laboratory procedures; and about half reported spending between 26 to 75% of their time performing hands-on laboratory procedures. Respondents indicated the program demographics to be as follows (see table):

About half of the respondents (55.6%) indicated that physicians encourage the laboratory to have patient consultations. About 41% of laboratory-patient interaction is limited to basic interactions such as identifying patients or introducing yourself. The majority of laboratory personnel spend between 1 and 30 minutes of interaction per patient.

About half of respondents indicated the commitment to certification of personnel and demonstrated this commitment with financial support.

The majority of programs utilize full size incubators with a tri-gas

mixture. Gas is supplied in liquid form for nitrogen in the largest percentage of programs (37.1%). CO₂ supply is in the form of gas for the majority of respondents (88.6%). The majority of respondents culture at 37°C (86%) and supply humidity using distilled water, sterile water or sterile distilled water equally. Incubator quality control was reported as a mix of daily Fyrite® and occasional pH. The majority of respondents overwhelmingly report desired pH of culture media is 7.2 to 7.4 and that protein supplementation of culture media is achieved using some brand of “selected plasma protein” (55.6%).

About half of the programs utilize IVF labeled culture ware that is pre-tested; while an additional 33.3% uses non-IVF labeled culture ware that is tested in house. Approximately 1/3 of retrievals take place in a laminar flow hood that is on; 1/3 on bench top and 1/3 in an isolette. About 70% of program respondents utilize a HEPES/MOPS based medium for retrieval. The majority of programs (52.8%) do not perform additional quality control on purchased medium.

Culture ware is labeled with a variety of methods, the most popular being a marker (50%). The most common second identifier is the patient’s date of birth or medical record number. Despite the trend to stop using social security numbers about 14% of respondents reported using SS# as the second identifier.

Embryo grading takes place most often on D1; D3; and D5. The most common time frame for stripping from retrieval is 2 to 3 hours and ICSI occurring 3 to 4 hours from retrieval. Most embryo transfers take place using abdominal ultrasound and echogenic embryo transfer catheters. About 57% of embryo transfers occur in bicarbonate based medium and 43% in MOPS/

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# Additional Embryologists		# Andrologists		# Physicians		Annual Fresh Retrievals	
Option	% Selected	Option	% Selected	Option	% Selected	Option	% Selected
0	11.0	0	58.3	1	27.8	< 100	19.4
1	38.9	1	33.3	2 to 4	44.4	100 - 200	25.0
2 - 3	27.8	2 - 3	2.8	5 to 8	16.7	201 - 400	22.2
4 - 5	11.1	4 - 5	2.8	> 8	11.1	401 - 800	22.2
5 - 7	5.6	> 5	2.8			> 800	11.1
> 8	5.6						

HEPES based medium. Greater than 50% of respondents report that the majority of their embryos transfers are blastocyst(s) transfers with the number of embryo(s) for transfer established after embryo evaluation.

The majority of programs function at 20 to 25°C (room temperature) with some type of air filtration system integrated in the HVAC system. About 36% of laboratories control the humidity within the laboratory.

Vitrification is the primary method of cryopreservation in 27.8% of respondent programs. Blastocoele collapse is applied prior to vitrification about 22% of the time. The majority of respondents use a closed system for vitrification.

Paper requisitions are the most common order format and AAB is the most common supplier of PT testing.

The survey is currently still open and the percentages currently reported are subject to change with additional input from additional laboratory directors.

Validation Of A Commercial Assay For Detection Of Vascular Endothelial Growth Factor In Clinical Specimens

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Background: Vascular Endothelial Growth Factor (VEGF) is a 34 to 45kDa homodimeric protein with antiapoptotic, mitogenic, and permeability-increasing activities specific for vascular endothelial cells. Circulating VEGF levels have been reported to be useful in indicating

cancer progression and predicting prognosis. In addition, VEGF-targeted therapeutics has undergone extensive clinical testing and some drugs have been accepted clinically as adjuvant medicine in management of a number of malignant tumors. The VEGF assay may also have some value in monitoring anti-VEGF treatment. The aim of this study is to evaluate a commercial enzyme immunoassay kit for clinical application.

Method: The Quantikine® human VEGF immunoassay kit (R&D systems, Inc. Minneapolis, MN), which quantitatively detects human VEGF₁₆₅ and VEGF₁₂₁ isoforms was evaluated and performance characters were established using clinical specimens (human EDTA plasma). The accuracy was assessed by comparing the assay with a reference laboratory method.

Results: An acceptable correlation with the reference laboratory (R =0.98; Slope: 1.114; Intercept 4.90; Bias:18.94 pg/mL) was obtained. Compared with the reference laboratory, the percentage of agreement for positive samples and negative samples was 94.1% and 82.6% respectively (n=40). The estimated imprecision (CV) for samples with low, middle, and high VEGF levels was acceptable (within run 3.7-8.3%; between run 0-10.6%; within day 0-4.5%; total 5.3-11.8%). The assay is linear within the range 15.6-1998 pg/mL. The lower limit of detection (LLOD, 2SD of blank) was 7.3 pg/mL. Interference was not observed with hemoglobin at 2g/L, free bilirubin at 20mg/dL and conjugate-bilirubin at 20mg/dL.

Conclusion: The commercial VEGF assay produces reliable results and is clinically useful in detecting VEGF in human EDTA plasma.

Women Age ≤38 Who Underwent Embryo Cryopreservation During The IVF Cycle Have At Least 70% Chance For Live Birth

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Objective: To evaluate live birth (LB) rate/retrieval in women age ≤38 who underwent embryo cryopreservation during fresh IVF cycles and cumulative LB (CLB) rate when pregnancies from frozen embryo transfer (FET) are included.

Design: A retrospective study.

Materials and Methods: All consecutive retrieval cycles with embryo cryopreservation in women ≤38 (418 cycles, 366 women) were analyzed. All subsequent frozen embryo transfers (FET) were traced to their corresponding retrieval cycles. LB rate from fresh embryo transfers per retrieval and CLB rate after FET per retrieval were calculated. Patients with more than one pregnancy after single retrieval were counted only once.

Results: Among 418 retrievals, 183 achieved LB from fresh embryo transfer (43.8%, Table 1). Of 235 retrievals without pregnancy, 142 underwent 1st FET, resulting in 62 LB. Of 80 who did not conceive after the 1st FET cycle, 43 had embryos remaining and 22 underwent 2nd FET cycle, resulting in 9 LB. The CLB per retrieval was 58.6% after the 1st FET and 60.8% after the 2nd. If all patients used their FET options, the projected CLB rate/retrieval would be 68.4% after the 1st FET and 75.2% after the 2nd FET. Both actual and projected

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cumulative LB rates per retrieval were significantly higher than the fresh LB only ($P < 0.0001$).

Table 1. CLB rate per retrieval when FET cycles were included (Age ≤ 38).

	Number	LB	LB%	CLB Actual	CLB Projected*
Retrievals	418	183	43.8%	43.8%	43.8%
1st FET	142	62	43.7%	58.6%	68.4%
2nd FET	22	9	40.9%	60.8%	75.2%

*If all non-pregnant patients used their FET options.

Conclusions: Women age ≥ 38 who undergo embryo cryopreservation during the IVF cycle have over 70% of chance for at least one live birth when cryopreserved embryos are used. Frozen embryo transfer can contribute 42% of LB to the cumulative LB per retrieval. With a successful frozen embryo transfer program, the number of embryos/transfer can be limited to 1 or 2 for those women with good prognoses, thus greatly reducing the chance of high order multiple pregnancies.

Cilostazol Blocks Pregnancy In Swine: Animal Model

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Cilostazol (Pletal®), a selective phosphodiesterase three enzyme inhibitor (PDE3-I), has been prescribed to treat patients with intermittent claudication (IC) disease. Cilostazol is considered the safest of the PDE3-Is as it has the ability to counterbalance the undesirable effects of other PDE3-Is on heart and blood vessels through its unique feature of adenosine uptake inhibition by cells. Recently, Cilostazol has been tested on mouse oocytes and found to arrest the in vitro meiotic maturation for 48 hours. The inhibition of PDE3 results in an intracellular oocyte cyclic adenosine monophosphate (cAMP) increase that arrests the

meiotic maturation. The objective of the present work was to study the clinical significance of Cilostazol as a contraceptive agent for humans using swine as an animal model. The sows were checked for their estrus cycle for three cycles and only those with regular cycle were selected for this experiment, on their 4th estrus cycle. Those sows were divided into three groups each of six animals per group (control, low dose, and high dose group). The low dose group received 100mg Cilostazol, bid, while the high dose group received 200mg, bid. The control animals received only prune (vehicle control), bid. The drug was administered orally for 6 days before the estrus period and continued for 6 days after the estrus period. Two proven fertile boars were mated alternatively with each animal in each group during estrus days. The mating was confirmed by direct daily observations. The pregnancy rate was completely blocked in animals treated with low Cilostazol dose compared to control animals ($P < 0.01$) while no significant differences recorded between high Cilostazol dose and control groups. No significant differences were observed in heart rates among all groups. We are reporting here for the first time that Cilostazol can block the pregnancy effectively in swine at 200mg daily without effect on heart rate, a dose that is prescribed in humans with IC. The lack of contraception effect that was observed in the high dose group may be due to the adenosine uptake inhibition that antagonizes the inhibition of PDE3 enzyme. It is concluded from the present study that Cilostazol can be used as a non-steroidal contraceptive agent.