Abstracts Presented in 2010

Oral Presentations
Friday, May 14, 2010
3:15 p.m.-5:15 p.m.

Age Impact on Chromosome and Spindle Disorganizations in MI and MII Oocytes: Animal Model

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ABSTRACT

Most of the chromosome aneuploidies in embryos have their origin in female meiosis. Oocyte aneuploidies are increased with advancing age in human and these aneuploidies are associated with maternal aging and may result from non-disjunctions and meiotic errors. The objective of this work was to use mouse as animal model to study the effects of age on chromosome and spindle disorganizations of MI and MII CBA/CAj mouse oocytes. Mice were grouped to young, 2.5-3.5 months and old, 7-8 months. Swiss Webster mice 2-3 months old were also used as a control because they have low percentages (<10%) of chromosome and spindle organizations. The animals were superovulated and sixteen hours after HCG, the oviducts were excised in FHM medium and the oocytes were retrieved from ampullary region of oviduct. The oocytes were denuded from cumulus cells by treatment with 0.1% hyaluronidase. Normal mature (MII) and Immature (MI) oocytes were recovered from ampullae and ovarian follicles. The oocytes were placed in fixative solution for 30 min at 37°C. The oocytes were washed five times in block-wash solution (BWS) and kept for overnight at 4°C. The oocytes were incubated for 1h at 37°C with Beta-tubulin mouse monoclonal antibody and then washed five times each 10 min. The oocytes were incubated for 1h at 37°C in FITC-conjugated goat anti-mouse Alex flour 488 secondary antibody. The oocytes were washed in BWS three times (20, 10 and 10 minutes) at room temp. The oocytes were stained with Propidium Iodide and mounted on barrier slides. The slides were stored in dark at 4°C for at least 48h to maximize signal noise ratio of imaging in fluorescent microscopy. The chromosome disorganization in MI young oocytes was 34% (21/61) and in MII oocytes was 38% (28/74, P> 0.05). The chromosome disorganization in MI old oocytes was 39% (21/54) and in MII oocytes was 39%. The spindle disorganization in MI young oocytes was 16% (6/38) and in MII young oocytes was 25% (14/57, P>0.05). The spindle disorganization in MI old oocytes was 24% (7/29) and in MII old oocytes was 23% (7/31). The comparison of chromosome and spindle disorganizations in MI and MII oocytes in young versus old mice showed no significant differences between the groups. The correlation between chromosome and spindle disorganizations was significantly higher in both groups (P<0.05). The percentage of the MII oocytes was significantly higher (P<0.01) compared to MI oocytes in the young group while it was not significantly different in the old group which may indicate that the oocyte maturation mechanism was affected adversely in the old group. It was concluded from the results of this study that the positive correlation between chromosome and spindle disorganizations in the MI and MII oocytes may be used as an indicator for embryo aneuploidy. Old CBA/CAj mice when superovulated by PMSG-HCG resulted in a significantly higher ovulation rate of MI oocytes as compared to young group. It seems that this strain of mouse is a good animal model to study age effects on aneuplodies.

Do All Commercially Available Micro-Tools Perform Equally?

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We hypothesized that there are performance differences in ICSI pipettes. To evaluate our hypothesis ICSI and holding pipettes from three different commercial vendors were randomly assigned to IVF ICSI cases during the evaluation time period. All IVF ICSI cases were handled in the manner established by the routine protocols in our laboratory. All ICSI pipettes were used in conjunction with the same vendor's holding pipette. Micro-tools all had a 30^{o} angle and injection pipettes were all spiked. ICSI cases were performed by one of two highly experienced technicians using one of two Eppendorf micromanipulation stations.

Data was evaluated based on the percentage of injected human ova that demonstrated two pro-nuclei, the percentage of degenerative ova post-injection and positive β-hCG.

(continued on page 2)

Results:

	Vendor A	Vendor B	Vendor C	
# Patients	57	77	69	NS
Mean patient age	36.1	34.7	34.7	NS
Mean # of oocytes injected	10.6	12.8	9.9	NS
Mean # 2 PN	8.0	9.1	7.1	NS
Mean # of deg	1.0	1.5	0.5	NS
Positive β-hCG	44.2%	45.4%	53.8%	NS

Using Chi-square analysis, no significant differences (P=<0.6) were detected between the mean patient age, mean number of oocytes injected, mean number of 2 pro-nuclei formed, mean number of degenerative oocytes post-ICSI and patients with positive pregnancy tests. The number of patients randomized into each group was influenced by the availability of micro-tools from the manufacturer during the evaluation period.

Conclusion:

Using 2 PN formation and percentage of oocytes degenerative post-ICSI did not detect differences in commercially available micro-injection tools. The percentage of patients with positive β-hCG was similar among the groups. The selection of vendor should be based on availability, pricing and technician preferences which exist between the selected manufacturers. Implantation rate is being analyzed as data becomes available.

Does Artificial Shrinkage Of The Blastocoele Prior To Vitrification Improve Post Thaw Viability?

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There has been a steady increase in the number of publications relating to vitrification of human blastocyst embryos. Occasionally, but not always, artificial shrinkage (AS) of the blastocoele prior to vitrification is detailed as a procedural step. Articles referring to artificial shrinkage do not present well controlled data.

There are a variety of commercially available vitrification solutions and carriers currently available. None of the commercially available vitrification systems mention artificial shrinkage of the blastocoele as part of their procedural steps.

Using a mouse embryo model, we evaluated artificial shrinkage using a commercially available FDA approved closed vitrification system (Irvine Scientific Vit-Kit). Frozen two-cell mouse embryos were thawed according to the manufacturer's direction and cultured to the blastocyst stage in cleavage medium (Quinn's Protein Plus Cleavage).

Blastocysts were randomly assigned to one of six treatment groups: All blastocysts were measured using the tool kit function of the Zilos TK laser to estimate the blastocoele volume. Blastocysts that underwent vitrification were de-vitrified using the same manufacturer's blastocyst thaw kit. Post de-vitrification, all blastocysts were cultured for 4 to 6 hours prior to fixation. All blastocysts were fixed for counting the number of cells within the blastocyst.

	Without Vitrification	Vitrification
NO AS (control)	TRT 1	TRT 4
AS With Laser	TRT 2	TRT 5
AS With Pipette	TRT 3	TRT 6

Results:

Our results indicate that AS using a single laser pulse does not decrease the blastocoele volume significantly, while AS using a fine boor pipette does. The results also

	TRT 1 N=30	TRT 2 N=30	TRT 3 N=30	TRT 4 N=30	TRT 5 N=30	TRT 6 N=30	P Value
Blastocoele Volume	71.75 ± 9.2[A]	57.56 ± 18.82[A]	64.35 ± 15.12[A]	66.70 ± 17.60[A]	68.57 ± 17.39[A]	70.41 ± 12.67[A]	NS
Blastocoele Volume Post Collapse	-	42.03 ± 15.62[B,C]	24.42 ± 19.93[D]	-	50.74 ± 21.64[B,C]	31.69 ± 25.49[D]	B = NS C = 0.017 D = 0.04
% Survive De-vitrification	-	-	-	93.94%[E]	93.94%[E]	86.67%[E]	NS
Mean Cell Number	66.12 ± 15.9[F]	53.56 ± 16.09[F]	52.27 ± 13.18[F]	64.92 ± 14.33[F]	51.64 ± 22.71[F]	53.33 ± 11.20[F]	NS

(continued on page 3)

indicated no advantage of AS of the blastocoele prior to vitrification as evidenced by the survival rate upon de-vitrification and overall blastocyst cell number in the mouse model.

Mitochondrial DNA Copy Number Is Significantly Increased In Aneuploid Relative To Euploid Human Embryos

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Appropriate mitochondrial content is critical to numerous cellular processes including normal metabolism, induction of apoptosis, and regulation of reactive oxygen species. Human embryo development is particularly dependent upon each of these processes. For example, increased reactive oxygen species within the embryo may play a role in the development of DNA damage and genomic instability. We hypothesized that mitochondrial content is associated with development of aneuploidy, the most common genetic abnormality observed in human embryos. Until now, simultaneous analysis of 24 chromosome aneuploidy and mitochondrial DNA content has not been performed. The present study developed an accurate method of single cell mitochondrial (mt) DNA copy number quantification using a combination of whole genome amplification (WGA) and real time PCR. The average quantity of 11 mitochondrial genome encoded genes (MT-7S, -ATP6, -ATP8, -CO1, -CYB, -ND1, -ND2, -ND4L, -ND5, -ND6, and -RNR1) was normalized to the quantity of a multicopy nuclear genome encoded gene, AluYa5. Accuracy of relative quantitation was established by evaluating quantities in single cells after WGA and then comparing the results to those obtained using isolated total DNA (without WGA) from the same cell line and culture dish. Ethidium bromide was used to inhibit mitochondrial biogenesis during multiple passages of the cell line. Single cell based analysis resulted in observing a similar relative decrease of mtDNA copy number (in response to ethidium bromide treatment) as that observed by analysis of isolated total DNA (Pearson correlation coefficient of 0.99). This validated technology was then combined with a previously established methodology for embryonic aneuploidy screening. Specifically, a portion of each embryo biopsy WGA DNA sample was evaluated for mtDNA copy number by real time PCR while a second portion was evaluated for aneuploidy by SNP microarray based 24 chromosome copy number analysis. WGA DNA was evaluated from blastomeres and trophectoderm biopsied from morphologically normal human embryos. Paired analysis of mtDNA copy number was performed on aneuploid embryos relative to euploid embryos derived from the same patient (n=10). This paired design allowed for the most precise control over all patient specific variables, isolating the differences among embryos to the greatest degree. Aneuploid and euploid embryo morphology characteristics, including fragmentation, weren't significantly different. Results demonstrate that mtDNA is significantly increased in aneuploid relative to euploid human embryos at the cleavage stage (P=0.02) but not at the blastocyst stage (P=0.46) of development. These results suggest that increased quantities of mitochondria may impact the development of aneuploidy in early embryogenesis.

Randomized Comparative Analysis Of Life Global® (LG) Medium And Vitrolife (G5) Series™ Medium

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Introduction: LG® medium formulated with glycylglutamine, was previously proven to be a reliable high performance BL culture system (Schiewe et al., 2009, AAB). The reformulated G5 Series™ has also been shown to be an excellent BL culture system. The aim of this study was to compare differences in blastocyst development and pregnancy outcomes between the two culture treatments, under low O_2 (5%) conditions, and to delineate the potential benefit of using paraffin oil (Ovoil™).

Material and Methods: In 2009, 90 patients from our center were enrolled in a randomized comparative trial to determine if we could improve clinical outcome with G5 Series™. Patients were sorted by age and randomly assigned to LG or G5 Series™ the day before OPU. Group A contained patient oocyte sources ≤34 y.o. with >10 follicles. Group B included patients 35-40 y.o. with >10 follicles. Cumulus oocyte complexes (COCs) were temporarily held in P1 (LG) or GIVF (G5 Series™) medium + 5% HSA until ICSI was performed. Post-ICSI, all oocytes were cultured in either LG medium + 7.5% SSS or G5 Series™ + 10% SSS in 25 µL droplets in 60 x 15 mm Nunc culture dishes under light mineral oil (7.0 ml Global® or Ovoil™, respectively). Tri-gas mini Sanyo (MCO-5) incubators were used, varying the $\%CO_2$ each lot to achieve the desired media pH for LG 7.31-7.35 and G5 Series™ 7.25-7.29. Normal 2PN zygotes and viable unfertilized eggs were isolated into a new culture dish on Day 1 (LG/G1)

(continued on page 4)

and on Day 3 (LG/G2) following embryo morphology evaluations. Embryo transfers were performed on either Day 3 or Day 5 by transvaginal ultrasound guidance and by use of Sureview™ Wallace® catheters. In a secondary experiment, we cross analyzed the effect of Ovoil™+ LG medium.

Results: Pregnancy outcomes between the two media across the two age groups (see Table below). Chi-squared analysis showed patients in LG had a significant increase in BIO/SAB rates despite having more ($P<0.05$) good-excellent quality BLs produced. This study revealed no statistical difference with pregnancy rates between the treatment groups in patients 35-40, yet an increase in ongoing pregnancy rates (OPR) did occur in patients ≤34 using G5 Series™ medium. In experiment II, higher ($p<0.05$) BL development occurred in LG using Ovoil™ with corresponding high pregnancy rates.

Conclusion: The use of G5 Series™ + Ovoil™ overlay may provide a reduction in BIO/SAB rates resulting in an increased OPR. Ongoing analysis of the LG culture system with Ovoil™ should help determine if the choice of oil influenced the increased BIO/SAB rates of the LG group. To date, results indicate that a comparable pregnancy rate to G5 Series™ + Ovoil™ can be achieved when adding Ovoil™ into the LG culture system. However, several more months of pregnancy observation is necessary to determine if a significant reduction in BIO/SAB rates has been achieved using Ovoil™ or if the LG medium itself contributed to the increase in BIO/SAB rates.

Group A

Pt. Age	medium	#Pts	μ#/BLET	+β-hCG (%)	Clinical preg. (%)	Ong. Preg (%)	#BL AA-BB (%)*
≤34	G5	26	2.1	20 (77)	20 (77)[a]	19 (73)[a]	142/285 (50)[a]
≤34	LG	24	2.0	17 (71)	13 (54)[b]	11 (46)[b]	149/241 (62)[b]

* BL development = BL ET + BL cryo (Blast ETs only); [a,b] column values are different ($P<0.05$)

Group B

Pt. Age	medium	#Pts	μ#/BLET	+β -hCG (%)	Clinical preg. (%)	Ong. Preg (%)	#BL AA-BB (%)*
35-40	G5	17	2.4	11 (65)	9 (53)	8 (47)	58/149 (39)
35-40	LG	23	2.4	16 (70)	13 (54)	11 (48)	86/203 (42)

Experiment II

Pt. Age	medium	#Pts	μ#/BLET	+β -hCG (%)	Clinical preg. (%)	Ong. Preg (%)	#BL AA-BB (%) *
≤34	LG + Ovoil	33	2.0	27 (82)	24 (73)	22 (67)	297/418 (71)[a]
35-40	LG +Ovoil	28	2.6	20 (71)	19 (68)	18 (64)	95/162 (59)[b]

Reduced Concentrations Of Zwitterionic Buffer In IVF Handling Media Maintains pH Buffering And Supports Mouse Blastocyst Development

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Inclusion of zwitterionic buffers in IVF handling media for use in procedures outside the laboratory incubator has provided more stable pH conditions to benefit gametes and embryos. However, though several studies have validated safety and efficacy of zwitterionic buffers, some of the predicate literature does suggest some detrimental effects. Importantly, these negative impacts appear to be largely reliant on buffer concentration. Therefore, reducing the buffer concentration used for IVF handling media, while maintaining appropriate pH and buffering capacity may be beneficial. Using a modified HTF with 4mM bicarbonate as a basal medium, we examined the ability various concentrations of the zwitterionic buffer HEPES (10, 12, 14 and 16mM) to maintain stable media pH in room atmosphere over a 1 month time period at various temperatures (10-12°C, 23-25°C and 33-35°C) in comparison to control media containing 21mM HEPES. Appropriate osmolality of all media were maintained through adjustment of NaCl. All concentrations of HEPES

(continued on page 5)

examined maintained stable pH over 1month at all temperatures examined, with no treatment varying more than 0.04 pH units compared to day 1 pH. In addition, 1ml of various HEPES buffered solutions (10, 12, 14, 16, 21mM) were incubated 24h in a sealed test-tube with varying concentrations of sperm (29 – 46 x 10^6 sperm/ml) at 37°C and pH measured. Data were collected over three replicates and ΔpH over 24h were compared. ΔpH data is presented as a mean ± SEM difference compared to 21mM controls, which were normalized to 0. Reduced concentrations of HEPES buffer resulted in a greater decrease in pH over time compared to 21mM controls (ΔpH 10mM: -0.09±0.02, 12mM: -0.07±0.03, 14mM: -0.06±0.01, 16mM: -0.03±0.01). Finally, ability of media to support mouse blastocyst formation from frozen-thawed 1-cell embryos was examined. Groups of 10 embryos were cultured in 500ul of respective media + 10% SSS and covered with 300ul of oil at 37°C in room atmosphere. Embryos were gassed twice daily with 5% CO_2 1h to satisfy metabolic requirements. pH of all media were maintained at 7.25±0.05. Data were collected over 3 replicates. No differences in cleavage rates were apparent at 8 or 24h No differences in cleavage >2-cell at 28h or rates of compaction at 48h were observed. No differences in early blastocyst formation were observed at 72h and no differences in total blastocyst formation or hatching rates were apparent at 96h between treatments. Total blastocyst cell number was also compared, with no significant differences were observed between treatments. In conclusion, utilization of reduced buffer concentration in IVF handling media is not detrimental to pH stabilization and is sufficient to support embryo development at room atmosphere.

Shortening Time Slow-Freezing Program for Human Embryo Cryopreservation

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Introduction: Embryo cryopreservation plays a significant role in assisted human reproduction. Currently, human embryos may be successfully frozen by traditional slow freezing or vitrification method. Although more and more reports have concentrated on vitrification technique, the traditional slow freezing method has still been widely applied and obtained satisfied result in many human IVF centers. The major disadvantage of slow freezing method is wasting time and liquid nitrogen. The objective of this study is to change cool rate of the slow freezing program so that the freezing time is shortened.

Materials and Methods: Traditional slow freezing method was performed as our laboratory standard procedure following: propandiol (PROH), as the cryoprotectant was used four step concentrations (0.5, 1.0 and 1.5M PROH and 1.5MPROH plus sucrose for embryo loading). All embryo loading procedures were performed at room temperature. One to five embryos were loaded in each 0.25 ml straw (Cryo-Bios, France). The straws were placed into Planer freezing control machine with temperature control program at the room temperature to be reduced at -2°C/min to -7°C. After 5 min the manual seeding was carried out. Ten minutes after seeding, the straws were cooled at -0.3°C/min. When temperature reached -35°C, the temperature was dramatically dropped to -140°C at -50°C/min for holding 2 minutes, and then plunged and stored in liquid nitrogen at -196°C. The modified method was the same as all procedure except for changing cool rate at -0.5°C from -0.3°C. Embryos were thawed as our standard procedure. The consuming liquid nitrogen was recorded for each freezing cycle. A total of 252 patient Day 3 (4-8 cell stage) embryos were randomly divided into two groups with two freezing methods. After freeze-thawing, embryos were evaluated and transferred.

Result

Our result indicated that shortening time slow freezing program had the same embryo survival rate, pregnancy rate as the traditional slow freezing method, but the our modified freezing program significantly shortened the freezing time to about one hour from previous near two hours. Also, the shorten time freezing program significantly reduced liquid nitrogen use.

Conclusion: The traditional slow freezing method could be improved by adding cooling rate to shorten freezing time and saving liquid nitrogen cost.

	Standard freezing method	Modified freeze method	Comment
Program running time	112.0 ±1.0 minutes	66.0 ± 0.5 minutes	P < 0.05
Consuming liquid nitrogen	8.5 ± 0.5 lb	6.5 ± 0.4 lb	P < 0.05
Embryo survival rate (good quality embryo on Day 3)	85/90 (94.4%)	84/90 (93.3%)	P > 0.05
Embryo survival rate (good quality embryo on Day 3)	42/80 (52.5%)	45/82 (54.9%)	P > 0.05
Transferable embryos #	3.175	3.0	P > 0.05
Clinic Pregnancy rate	17/40 (42.5%)	18/43 (41.8%)	P > 0.05

(continued on page 6)

The Assay Time of Human Sperm Bioassay Used in Proficiency Testing for Assisted Reproductive Technology Laboratories are Unnecessarily Lengthy

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Objective: Fertility laboratories routinely use human sperm bioassay (HSB) as an in house quality control (QC) test and also as an externally administered proficiency test (PT). The American Association of Bioanalysts (AAB) has been the largest PT provider for fertility laboratories for more than 10 years. In AAB administered PT, it is mandatory to perform sperm assays for 48 hours for the participating laboratories that use HSB for acceptability determination of embryo culture media. In the present study, we evaluated the AAB compiled PT data on HSB, and also performed additional HSB experiments to see if such prolonged time (48 hours) is really required to achieve the goal.

Study Design: Differences between media of acceptable quality (MAQ) and media of unacceptable quality (MUQ) were assessed using AAB generated HSB data, and our own HSB data collected sequentially over 48 hours.

Materials and Methods: AAB compiled PT scores for HSB, of more than 130 participating laboratories for years 2008 and 2009, were used as the data. These laboratories employed HSB for determining the suitability of two culture media, labeled as embryology culture 1 and 2 sent to them twice each year. The laboratories were required to establish the culture with and without protein, either under oil overlay or without oil overlay. AAB required that the participating laboratories record the sperm motility in the culture system constituted of the supplied media at 0 hr, 24 hr and 48 hr time points in order to differentiate the MAQ from MUQ. In addition, we performed HSB on the AAB provided media (event 1, 04-2009) in which sperm motility was evaluated sequentially at 0, 2, 4, 6, 8, 10, 12, and 24 hours time points. The minimum culture duration to differentiate MAQ from MUQ was determined based on AAB compiled HSB data and also from our own HSB data.

Results: Approximately 25% and 75% HSB using laboratories utilized oil overlay and no oil overlay respectively. The motility-drop rate under oil overlay was higher compared to that with no oil overlay in both MAQ (17.5 vs 12.3 at 24 hr; 24.4 vs 15.2 at 48 hr) and MUQ (53.9 vs 42 at 24 hr; 40.8 vs 23 at 48 hrs). As expected, sperm in MUQ lost motility faster compared to that in MAQ. The motility difference between MAQ and MUQ was statistically significant at 24 hour ($p \leq 0.05$) as well as at 48 hour ($p \leq 0.05$). The significant difference ($p \leq 0.05$) between MAQ and MUQ during the first 24 hours was found in all four PT events (2008-1st, 2008-2nd, 2009-1st and 2009-2nd) irrespective of methods of culture (oil overlay and no oil overlay) and protein supplementation (with and without protein). In our own HSB experiment with AAB provided culture media, the quality of sperm motility (motility grade) in MUQ was distinguishable from that of MAQ by 4-8 hours while the motility (% motile) difference between MAQ and MUQ was identifiable from 12 hrs (86% vs 75%) onward.

Conclusion: AAB compiled data suggests that sperm culture beyond 24 hours is not necessary to differentiate the two culture media. Our own HSB indicated that an assay time of about 12 hours is likely sufficient for confidently indentifying MAQ and MUQ in a properly arranged experimental setup.

Poster Presentations
Friday, May 14, 2010
6:15 p.m.-7:30 p.m.

A Novel Stain For Renal Biopsies For Better Evaluation Of Interstitial Fibrosis

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Histologic evaluation of renal biopsies includes multiple ancillary stains, including Periodic acid-Schiff's (PAS) and Masson's trichrome (Trichrome). These ancillary stains have been used in the renal pathology practice for many years and each of them yields additional information, important for the final pathologic diagnosis. PAS stain is used to highlight basement membranes, including the glomerular basement membrane (GBM) and tubular basement membrane (TBM) in dark-red to purple. Trichrome stain stains all collagens, including those which compose basement membranes, blue. Therefore, determining the degree of interstitial fibrosis based on Trichrome stain often results in overestimation. Quantitation of interstitial fibrosis is very important in assessing the efficacy of drugs in clinical trials, as well as progression of chronic kidney diseases in native and transplant kidney biopsies. Herein we report an innovative double-stain, derived from two standard stains (PAS and Trichrome). This innovative double-stain has advantages of both PAS and Trichrome stains and significantly increases the amount of useful information, which could be obtained from one slide. First, each section stained with this double-stain has an extra dimension added by the combination of two individual stains. Second, in the new stain, basement membranes stain dark-violet, as a result of the combination

(continued on page 7)

of blue (Trichrome) and purple (PAS). The interstitial collagen remains blue, because it is only faintly PAS positive. This allows a pathologist immediate estimation of the amount of collagen and the degree of fibrosis in one section, without spending time for evaluation of two different stains. This is especially important when non-consecutive sections are stained. Third, certain deposits (e.g. amyloid) became more distinguishable in the double-stained sections. Using computer-based analysis, we confirmed that our innovative double-stain highlights interstitial collagen better than Trichrome stain alone. This novel stain has been used in our routine practice for a several months. We did not loose any of the important features of Trichrome stain alone, but we gained the ability to easy evaluate the degree of interstitial fibrosis and tubular atrophy in renal biopsies. We strongly recommend renal pathologists to try this innovative stain.

Characteristics of Assay Controls of Different Hormone Assays Performed in Fertility Laboratories

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Objective: Hormone assays are an integral part of an infertility clinic. Clinical laboratories perform assays on patients' hormone levels that are directly or indirectly related to human reproduction. These assays are used for infertility diagnosis as well as gonadotropin induced ovulation induction. Running a hormone assay was a very cumbersome task in the recent past. However, with the advancement of technology, the hormone assays have now become automated. Since hormone assays are now mostly commercial kit based, laboratory personnel are no longer required to prepare reagents and supplies to run an assay. The kit contains assay control (control sample) to assure that every run of an assay by the machine is reliable. In the present study, we evaluated the reliability of the manufacturers' provided controls of different hormone assays performed for infertility patients.

Study Design: The lot specific control values obtained in the laboratory (observed values) during patients' sera testing for several different reproductive hormones were evaluated and compared with those of the expected values (values provided by manufacturer).

Materials and Methods: The fertility laboratory at University of Texas medical branch performed hormone assays for its infertility patients using assay kits from bioMerieux Vitec, Inc. using an assay machine also provided by the same company (bioMerieux). Nine hormone assays (FSH, LH, E_2, P_4, HCG, TSH, FT_4, Prolactin and Testosterone) were performed. Values obtained from the control samples run for each of these assays were used as the data. The lot specific pattern of distribution of the control in each assay was evaluated. Different measures of central tendency of the observed values were determined and compared with that of manufacturers' provided values.

Results: The hormone assays that were run in the fertility laboratory can be divided into two groups based on how frequently each assay was ordered. The most frequently ordered assays were E_2, HCG, FSH, and P_4 while LH, TSH, FT_4, Prolactin, and Testosterone can be considered as the less frequently ordered assays. Observed control values fall within the manufacturers' range in all assays except testosterone 98% of the time. Testosterone, LH, TSH, P_4, and FSH had a highly narrowed (<2 units) control range. The widest range (>20 units) was for E_2 assay. The observed range (laboratory values) was always shorter than the expected range (bioMerieux values) in all assay lots. The coefficient of variation (CV) was relatively high (15-20%) in P_4 and testosterone while it was around 10% in other assays (E_2, HCG, FSH, LH, TSH, FT_4 and Prolactin). The highest (20%) and lowest (2.5%) CV were recorded for the Testosterone assay and TSH assay respectively. The observed mean, median, and mode in each lot of assays were closely identical suggesting a symmetrical distribution of the control values in a lot.

Conclusion: Hormone assay controls provided by bioMerieux were consistently reproducible in the hormone analyzing machine. The unshakable stability of the assay controls indicates that running one control per day instead of including a control for every run in the day may be cost effective without compromising the test accuracy.

Cryopreservation Of Biopsied Multicellular Embryos Followed By Day 4 Embryo Transfer Does Not Reduce Pregnancy Rates in Egg Donation Cycles

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Introduction: Day 4 embryo transfers have been shown in several studies to result in comparable implantation and pregnancy rates to day 3 and day 5 transfers. Implementing day 4 transfers into an in-house preimplantation genetic screening (PGS) for aneuploidy program permits greater scheduling flexibility and allows selection of morphologically

(continued on page 8)

high quality euploid pre-blastocyst stage embryos.

Objective: To compare clinical outcomes after day 4 transfers in fresh versus frozen egg donation cycles, and to evaluate whether blastomere biopsy prior to freezing is detrimental to the cryosurvival of multicellular-stage embryos.

Materials and Methods: A retrospective study of data reviewed from egg donation cycles undergoing PGS with day 4 embryo transfers. Biopsy was performed on day 3 and embryos cryopreserved by controlled rate slow freezing. Cycles were divided into 3 groups: Group 1 were fresh transfers (n=46), Group 2 were frozen transfers of embryos biopsied prior to day 4 freezing (n=27), and Group 3 were frozen transfers of embryos frozen on day 2 or 3, thawed and subsequently biopsied (n=20). Gender selection was a factor in 30% of cases in each group and overall euploidy rate was 549/842 (65%) with 5-probe FISH.

Results: The number of embryos transferred (mean: 2.2) and percentage that were undergoing compaction or were fully compacted (mean: 72%) was not significantly different among the three groups. Clinical pregnancy rates were comparable in the three groups (87%, 81% and 90%) and ongoing pregnancy rates were not statistically different (Group 1: 80%, Group 2: 63% and Group 3: 85), although trending lower in Group 2. Implantation rates were also not significantly different between the 3 groups (61%, 47% and 57%); however, there was a trend toward higher rates in the fresh cycles as expected, since no prior embryo selection had taken place. Very similar post-thaw survival rates (>60% cells intact) were obtained with biopsied versus non-biopsied embryos (group 2: 83%, mean number thawed 3.3; versus group 3: 86%, mean number thawed: 7.1), and the percentage of embryos that survived with no cell lysis was comparable (61% versus 69%).

Conclusions: A successful slow-freezing protocol for biopsied cleavage-stage embryos allows high frozen pregnancy rates comparable to those achieved in fresh cycles. Having the option to perform PGD before or after cryopreservation and to transfer embryos prior to day 5 is particularly useful in mitigating the scheduling issues that frequently arise in third party reproduction cycles.

Reconstruction Of An Assisted Reproductive Technology (ART) Laboratory Following A Major Water Leak

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Objective: To describe our emergency response to a large water leak in our ART laboratory and the resulting reconstruction of the laboratory.

Setting: Hospital-based ART practice.

Results: Despite a quick response by laboratory and hospital personnel to a water leak in the ART Lab and adjacent ART Operating Room, both areas had to be completely renovated before being fit for use for patient care.

Conclusions: Personnel in each ART laboratory should develop an emergency response plan suited to their own facility. Our emergency situation required the coordinated efforts of laboratory and hospital personnel from several departments to achieve a successful resolution.