2015 AAB Conference and CRB Symposium

Abstracts

May 14-16

Red Rock Resort
Las Vegas, Nevada

ABSTRACTS – ORAL

ABSTRACTS – POSTER

An Elective Single Blastocyst Transfer Derived from Early Rescue ICSI with Application of Polarized Light Microscopy for Spindle Examination

Moon, J.H.; Garcia-Cerrudo, E.; Henderson, S.; Buckett, W.; and Son, W.-Y.
McGill Reproductive Center, McGill University Health Center, Quebec, Canada

Objective: We aim to report the outcome of a single blastocyst transfer after early rescue ICSI using the Polscope^{TM} to examine spindle on unfertilized eggs.

Design: Prior to rescue ICSI, unfertilized MII eggs' spindles were examined with the Polscope^{TM} in order to select those with one spindle that failed to fertilize due to non-penetration of sperm and exclude those with two spindles that resulted from incomplete egg activation with sperm positioned inside. Early rescue ICSI with Polscope^{TM} can help to not only decrease 3PN formation but also save embryo development potency by reducing aging.

Materials and Methods: From Jan. 2013 to Dec. 2014, 11 women (female mean age ± SD; 32.8 ± 4.6) with total fertilization failure (N=5) or low fertilization rates (<20%) (N=6) following conventional IVF were included. At the time of fertilization assessment following conventional insemination (16-18 h), unfertilized eggs (N=120) were examined for spindle presence by Polscope^{TM} prior to rescue ICSI. Eggs with only one spindle and one PB promptly had rescue ICSI performed. Eggs with two spindle positions did not undergo rescue ICSI. Fertilization assessment of rescue ICSI was performed in the afternoon of same day (~ 6 h after ICSI). 2PNs were cultured until Day 5 or 6 and a single blastocyst was transferred into the patient's uterus and freezable blastocysts were vitrified for further use.

Results: A total 107 (89.2%) unfertilized eggs showing one spindle had rescue ICSI performed whereas 13 (10.8%) eggs showed two spindles and were excluded. After rescue ICSI, 83 (77.5%) eggs out of 107 showed evidence of normal fertilization (2PN) and only 3 (2.8%) showed 3PN formation. A total of 23 (27.7%) embryos reached blastocyst stage after culturing until Day 5 or 6 and 14 (60.8%) out of 23 were vitrified after embryo transfer. Five patients with total fertilization failure following conventional insemination had a single blastocyst transfer derived from rescue ICSI resulting in three pregnancies and one ectopic pregnancy. However, this patient returned for a frozen-thaw cycle which resulted in a successful pregnancy.

Conclusions: Embryos obtained from early rescue ICSI could develop to good quality blastocysts leading to a successful pregnancy within same cycle while avoiding cancellation. Additionally, this provides patients with another opportunity for a frozen-warmed blastocyst cycle as well.

Disclosures:

Funding:

Clinical Pregnancy and Implantation Rates of Warmed, Biopsied and Re-vitrified Blastocysts (W-CCS)

Popwell, J.M., and Conaghan, J.
Pacific Fertility Center, San Francisco, California

Background: Comprehensive chromosome screening (CCS, PGS) is being implemented by IVF practices as risks associated with embryo biopsy decrease and outcome data continue to improve. CCS prior to embryo transfer may improve implantation and pregnancy rates. With the progression towards Day 5 embryo culture and elective single embryo transfer, many patients cryopreserve excess embryos. Performing CCS on previously cryopreserved embryos could also benefit patients considering frozen embryo transfers (FETs).

Objective: The purpose of this study was to evaluate clinical pregnancy and implantation rates from patients with CCS performed on previously vitrified blastocysts.

Design: Retrospective study in a private assisted reproductive technology program.

Materials and Methods: A 2013-2015 database review identified cases of CCS performed on blastocysts warmed prior to trophectoderm biopsy ("W-CCS"). All blastocysts had been previously vitrified from 2007-2014 using a vitrification kit and cryotips. Blastocysts were warmed using a warming kit and biopsied two hours post expansion. Post-biopsy, trophectoderm samples were placed in microtubes for shipping and blastocysts were collapsed and re-vitrified in cryotips. CCS was performed offsite (Natera, IviGen, and Genesis). FETs of euploid blastocysts were later performed following natural or controlled patient stimulation.

Results: From 2013-2015 there were 33 cases of W-CCS where 84% of blastocysts warmed were biopsied and 68% were euploid. The median age of the patients at first blastocyst vitrification was 36 years. Twenty-five FETs were performed (ten cases remain frozen; four cases (100% aneuploid). 29 blastocysts were re-warmed, 97% survived and 93% were transferred. The clinical pregnancy rate was 72% and the implantation rate was 60%. The delivery rate was 50% (8 cases pending). Pregnancy and implantation rates were comparable to our overall 2013 Clinic rates for IVF/FET using CCS techniques (64%, 65%, and 56% respectively) with fresh blastocysts (chi square: p=0.63 and p=0.43).

Conclusions: These data demonstrate CCS has the potential to be performed on previously vitrified blastocysts (W-CCS) with positive clinical outcomes. Transferring euploid blastocysts screened following W-CCS yielded pregnancy and implantation rates comparable to CCS performed on fresh blastocysts. Knowing the chromosome status of cryopreserved embryos may benefit clinicians and patients evaluating transfer and embryo disposition options.

Disclosures: None

Funding: None

Frozen Embryo Transfer of Vitrified Blastocysts Results in Significantly Higher Pregnancy Rates Compared to Fresh Blastocyst Transfers

[1]Picou, A.; [1]Hellmers, A.; [1]Werland, H.; [1]Turner, T.; and [2]Silverberg, K.
[1]Austin IVF
[2]Texas Fertility Center, Austin, Texas

Objective: To compare pregnancy rates of frozen (vitrified) blastocyst transfers to fresh blastocyst transfers.

Design: Retrospective study in a private assisted reproductive technology program.

Materials and Methods: Pregnancy rates for the transfer of vitrified blastocysts were compared with those of fresh blastocysts in 440 embryo transfer cycles. A total of 354 frozen embryo transfers (FET) and 86 fresh embryo transfers (ET) were performed from January 1 to December 31, 2014. All embryos were cultured in Continuous Single Culture media (Irvine Scientific) to the blastocyst stage and were vitrified on Day 5, 6 or 7 once they reached freeze quality (grade CC or higher). All fresh transfers were performed on Day 5. For freezing embryos and for transfer, the best quality embryos were selected. Blastocysts were collapsed prior to freezing. Vitrification and warming were performed using Irvine Scientific freeze and thaw kits and Cryolocks. Patients who used donor eggs were excluded from this study. The outcomes of interest were positive hCG and positive clinical pregnancy rates (defined as the presence of fetal cardiac activity at 7 weeks).

Results: A total of 253 of the 354 (71%) FET patients had a positive hCG compared to 48 of the 86 (56%) ET patients ($p < 0.05$). Of the FET patients, 182 (of 354) developed fetal cardiac activity at their 7 week ultrasound, resulting in 51% clinical pregnancy rates. In comparison, 37 (of 86) patients who had a fresh transfer developed fetal cardiac activity, resulting in 43% clinical pregnancy rate ($p < 0.05$).

Conclusions: Embryos vitrified at the blastocyst stage resulted in higher pregnancy rates and clinical pregnancies compared to fresh blastocyst transfers. These results indicate that vitrification and subsequent frozen embryo transfer of high quality blastocysts results in significantly higher clinical results than the transfer of fresh blastocysts.

Disclosures: Nothing to disclose

Funding: None

Impact of Different pH on Mouse Embryo Development

[1,2]Youssef, A.; [2]Kandil M.; [2]Makhlouf, A.; [1]Mesiano, S.; [1]Liu, J.; and [1]Ahmady, A.
[1]Departments of Reproductive Biology, Case Western Reserve University, Department of Obstetrics and Gynecology, University Hospitals of Cleveland, Cleveland, Ohio, USA
[2]Department of Obstetrics and Gynecology, Women Health Hospital, Assiut University, Assiut, Egypt

Objective: To determine the optimum pH for mouse embryo development.

Design: We postulate that pH will have significant impact on systems that regulate embryo development. One cell mouse embryos were cultured in media at two physiological pH concentrations for 5 days and effects of pH on embryo development were determined.

Materials and methods: Cryopreserved one cell mouse embryos (n=200) were thawed in G-MOPS PLUS (Vitrolife) media under oil for tissue culture (SAGE) then cultured for 5 days at 37.5°C in media at pH 7.3 (n=100) and 7.6 (n=100) in a CO_2 incubator. Embryos were cultured in groups of 5 to 10 in 50 μL drops of medium under paraffin oil (Vitrolife). Incubator temperature and media pH were measured daily, and the CO_2 level was adjusted to maintain the pH within the assigned range (± 0.1). Embryo development was monitored daily and scored morphologically for 5 days. Our primary endpoints were rates of blastocyst and hatching embryos. The chi-square test was used for comparison between the 2 groups, and P value < 0.05 was considered significant.

Results:

Day 5	pH 7.3 (n=100)	pH 7.6 (n=100)
Hatching	76	58
Blastocyst	16	23
Morula	4	-
Compacting Morula	1	3
Degeneration	3	16

The number of hatching embryos at pH 7.3 was significantly higher than at pH 7.6 ($P < 0.01$). The total number of hatching and blastocyst together at pH 7.3 (n=92) was significantly higher than at pH 7.6 (n=81) ($P < 0.023$). A significantly increased number of embryos degenerated at pH 7.6 ($P < 0.001$).

Conclusions: Culture of mouse embryos at 37.5°C and pH 7.3 is optimal for embryo development to the hatching alone and post-hatching to the blastocyst stage. Our model suggests that media at pH 7.6 increased the number of degenerating embryos and is not optimal for mouse embryo development.

Disclosures: Nothing to disclose

Funding: None

In Patients with Isolated Teratozoospermia, Blastocysts Derived from ICSI Have Better Morphology Than Those from Conventional Insemination

[1]Gray, J.E.; [2]Crawford, N.M.; and [1,2]Berger, D.S.
[1]UNC Fertility, Raleigh, NC 27617
[2]Obstetrics and Gynecology, Reproductive Endocrinology and Infertility, University of North Carolina, Chapel Hill, North Carolina

Objective: To evaluate if fertilization rates, embryo development, and pregnancy rates vary in intracytoplasmic sperm injection (ICSI) cycles compared with conventional in vitro fertilization (IVF) cycles in patients with isolated teratozoospermia.

Design: Retrospective study.

Materials and Methods: Women undergoing a fresh, autologous IVF and/or ICSI cycles using partner or donor sperm from 1/2013-1/2015 were included in the study. Patients were categorized as having isolated teratozoospermia as defined by < 4% normal forms by Kruger's strict morphology (1) and within the WHO 4th edition (2) reference ranges for volume, total sperm number, sperm concentration, percent motility, and progressive motility within the preceding year.

High quality blastocysts were defined as having expansion of 3 or greater and grades of A or B for both inner cell mass and trophectoderm by Gardner's grading system (3). Sample size was calculated based on an ability to detect 80% power. Comparisons between groups were performed using Pearson's chi square and Student t-tests for categorical and continuous variables, respectively, with a p value of <0.05 considered significant.

Results: A total of 3075 embryos from 329 cycles were examined. Of those, we identified 300 embryos from 26 cycles that fit criteria of isolated teratozoospermia. Pregnancy rates were not significantly different when comparing ICSI, 9/13 (69% pregnant), and conventional or split IVF/ICSI insemination cycles, 8/13 (61% pregnant). The production of blastocysts, high quality blastocysts, and vitrified blastocysts was improved by ICSI compared to IVF, p values < 0.01. See Table 1 below for results.

Table 1.	**ICSI** n (%)	**IVF** n (%)	p value
Cycles	19	13	
Mean Female Age	34.5	35.5	0.32
Oocytes Retrieved	261	210	0.17
Mature Oocytes	211	157	0.33
Normal Zygotes Day 1 (% of oocytes retrieved)	177 (68%)	123 (59%)	0.17
Blastocysts (% of zygotes)	114 (64%)	59 (45%)	<0.01
High Quality Blastocysts (% of zygotes)	93 (51%)	40 (26%)	<0.01
Vitrification (% of zygotes)	79 (40%)	31 (19%)	<0.01

Conclusions: Our study found that fertilization and pregnancy rates do not differ between IVF and ICSI for patients with isolated teratozoospermia. Formation of more high quality blastocysts and subsequently more blastocysts for vitrification was improved by ICSI when compared to IVF in our study of isolated teratozoospermic patients. This potentially suggests exposure of zygotes to large numbers of sperm from

In Patients with Isolated Teratozoospermia Blastocysts, Derived from ICSI Have Better Morphology Than Those from Conventional Insemination *(continued from page 6)*

teratozoospermic men may be deleterious to blastocyst development. Damaging factors produced by sperm, such as reactive oxygen species, could be mediating these effects and should be subjects of future study. Insemination via ICSI rather than IVF should be considered with isolated teratozoospermia as it allows patients greater opportunities for frozen embryo transfer cycles.

Disclosures: None

Funding: None

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Resiliency of Human Blastocysts to Cryotoxicity and Repeated Osmotic Stress Using a Re-Vitrification Model

Schiewe, M.C.; Gamboa, L.; Smetona, V.; Borba, J.; and Zozula, S.
Southern California Institute for Reproductive Sciences, Newport Beach, California

Objective: Vitrification (VTF) has proven to be a highly efficient mode of embryo cryopreservation. Survivability is dependent on 1) exposure to concentrated cryoprotective solutions that form a metastable glasseous solid state upon rapid cooling, and 2) high warming rates followed by cellular elution to an isotonic, equilibrated condition. We have previously shown that human embryos are highly adaptive to osmotic shifts during elution and that the re-VTF (rVTF) of blastocysts can serve as an effective research model. The aim of this study was to assess the cryotolerance of human blastocysts to repeated rVTF and determine the degree to which cytotoxicity of VTF solutions or osmotic membrane stress represents potential sources of cryoinjury.

Design: Using two 2x3 factorial arrangement of treatments, 160 research consented, discard blastocysts were randomly assigned to: Expt.1) either repeated rVTF (1, 3 or 5 times) with or without elution/equilibration; or Expt.2) extended rVTF exposure (1, 3 or 5 min) to either a DMSO-free, glycerol-based VTF solution (Innovative Cryo Enterprises) or a commercial EG/DMSO VTF solution (LifeGlobal). Differences in %survival and %24h development were statistically compared by chi-square analysis.

Materials and Methods: Standard microSecure vitrification (mS-VTF) and rapid warming of flexipettes (Cook, 300 μmID) was performed. In Expt.1, half the embryos were eluted in decending sucrose solutions (1.0M to 0.125M; 3 min/step) and equilibrated for 30 min before rVTF (with a controlled 1 min exposure to V3 solutions), while the other flexipettes (containing embryos) were directly dried with sterile gauze, reinserted into new straws and vitrified 1 min post-warming (i.e., non-elution). In Expt.2, mS-vitrified, warmed, eluted and rediluted blastocysts were exposed to final VTF solutions for 1-5 min before elution, equilibration and 24h embryo culture.

Results: Human blastocysts proved to be highly cryotolerate to extended VTF solution exposure (with or without VTF), dilutions/elutions and rVTF, with no differences in post-warming or developmental survival being observed in Expt.1 or 2 treatment groups. Overall, 97.5% survival of intact blastocysts and 94.8% sustained development was observed.

Discussion: Human blastocysts are highly resilient to extended exposures (≤6 min total) to VTF solutions, independent of the permeating cryoprotectants used. Routine production of healthy live births occurs following a single rVTF, but it is not known what genetic alterations could be associated with >5 min combined exposures to VTF solutions, if any. The safety and efficacy of VTF was clearly shown to be highly tolerable by the functional cell membrane integrity sustained after repeated metastable rVTF.

Disclosures: This study was conducted, in part, as a summer student training program research project (V. Smetona).

Funding: None

The GENETWORx Personal Health Panel: A Pharmacotherapy Guided Approach to Genital Pathogen Diagnosis and Treatment

Jacobs-Helber, S.; Beckman, M.; Miller, W.; and Blevins, S.
GENETWORx, LLC, Glen Allen, Virginia

Objective: To evaluate a urogenital pathogens molecular diagnostic panel that includes therapeutic recommendations for its utility in detecting the presence or absence of genital pathogens and to determine how rate of co-infection of pathogens found in different groups of pathogens affects the recommended treatment for infection.

Methods: A total of 659 deidentified patient results were evaluated. The GENETWORx Personal Health Panel is comprised of a comprehensive panel of 23 urogenital pathogens. The panel is composed of three leukorrhea (Leuko) strains, six bacterial vaginitis (BV) strains, six candida vaginitis (CV) strains, four ureoplasma/ mycoplasma strains (Urogen), Herpes simplex viruses (HSV) 1 and 2, and group B streptococcus (GBS) as well as 24 high-risk and 2 low risk human papilloma virus (HPV) strains. All tests were performed from a single swab collection shipped in universal transport media. Pathogen detection was performed using multiplex PCR followed by fluorescent primer extension and microarray hybridization. Therapeutic recommendations for treatment of positive patients were provided by pharmacists based on CDC and medical treatment guidelines. Rates of infection of each pathogen were determined as well as rates of co-infection and incidence of less common HPV strains.

Results: The frequency of detection for each pathogen fell within expected limits with regard to population and ethnicity. 68% (445/659) of patient reports evaluated were positive for at least one pathogen. Of those 445 positive patients, 47% (208/445) of patients were positive for more than one pathogen requiring differential treatment. The most common pathogens observed during co-infections was the bacterial vaginosis strains *Gardnerella vaginalis* (GV) and *Prevotella bivia* (PB); 63% of individuals infected with *Candida* species were also infected with GV and or PB, and 50% of individuals infected with *Trichomonas vaginalis* were co-infected with GV. Additional co-infection rates will be discussed. It is also of interest to note that of the 26 high risk-HPV positive patients, 65% (17/26) were not among the four more common high-risk HPV types screened (HPV16, 18, 31 and 45).

Conclusions: The use of a panel of urogenital health tests provides a more comprehensive view of the presence of the vaginal flora as compared to timely and individualized culture methods and provides more personalized treatment for individuals infected by one or multiple pathogens. This approach is of special interest in HPV detection which may detect high-risk strains absent in smaller screening panels.

Disclosures: None

Funding: None

Use of Comprehensive Chromosome Screening as Part of Routine Infertility Care is Associated with Decreased Transfer Order and Increased Elective Single Embryo Transfer

Franasiak, J.M.; Werner, M.D.; Juneau, C.R.; and Scott, R.T.
Rutgers, Robert Wood Johnson Medical School; Reproductive Medicine Associates of New Jersey

Objective: Multiple gestations due to multiple embryo transfer are the leading cause of maternal and neonatal morbidity related to infertility treatment. Many technologies have been employed to apply selective pressure to an embryo cohort with the hopes of selecting fewer embryos with greater reproductive potential and thus lowering transfer order. We sought to evaluate the impact of offering routine comprehensive chromosome screening (CCS) in the general infertility population on the number of embryos selected for transfer.

Design: Retrospective cohort study at a single, academic center.

Materials and Methods: The electronic medical record was utilized to collect data on all first time IVF cycles from 1/2012 to 12/2014 along with the first fresh or frozen embryo transfer cycle following oocyte retrieval. During this period of time CCS was routinely offered in this clinic to all patients as a way to increase pregnancy rates and decrease miscarriage rates. Data collected included use of CCS or not, number of aneuploid/euploid embryos if CCS was performed, number of embryos transferred, and number of embryos cryopreserved which was used to determine the rate of elective single embryo transfer in cases where there were embryos which were cryopreserved in excess of those transferred. Patients with known single gene cases or translocations were excluded as were study protocol cycles.

Results: During the study timeframe there were 3312 unique patients identified. Of these 1562 (47%) underwent preimplantation genetic screening with CCS and 1750 (52.8%) did not. Of those undergoing PGD, the average number of embryos biopsied was 5.4 (1-34), number euploid was 3.7 (0-27), number aneuploid was 1.7 (0-13), and 99 (6.3%) had no euploid for embryo transfer. Of the 3213 remaining, when CCS was employed the SET rate was 71.9% and DET rate was 28.1% compared to an SET rate of 43.0% and DET rate of 57.0% when patients did not elect CCS. There were no transfers which had more than 2 embryos put back. The mean number of embryos transferred was 1.3 with CCS and 1.6 without CCS ($p<0.0001$). The rate of elective single embryo transfer (eSET) was 19.1% in the CCS group and 15.7% in the group without CCS ($p=0.0095$).

Conclusions: When patients and physicians incorporate CCS into routine IVF care both the average numbers of embryos and the rates of eSET are increased. Given the significant maternal and neonatal morbidity associated with multi-fetal births, any intervention which decreases transfer order represents a significant advancement in infertility care.

Disclosures: None

Funding: None

A Rapid Non-Arbitrary Histomorphometric Method of Quantifying Ovarian Follicle Atresia in the Mouse

Uslu, B.; Dioguardi, C.C.; and Johnson, J.
Yale School of Medicine, New Haven, Connecticut

Determining the number of intact ('healthy') follicles in an ovary at any time is a central measurement in reproductive biology. If intact follicles can be calculated, the current reproductive status of the ovary, its relative ovarian 'age', and the impact of genetic or environmental modifiers upon follicle number can be understood. This can be done by the histomorphometric assessment of follicles in serial sections. Subtracting the number of follicles in an ovary that are undergoing death (termed atresia, 'atretic' follicles) from the total number reveals the number of intact follicles. A standard histomorphometric approach has been used for approximately 40 years that identifies atretic follicles by counting the number of pyknotic granulosa cell nuclei in the largest follicle cross-section. This method holds that if one pyknotic granulosa nucleus is seen in the largest cross section of a primary follicle, it can be categorized as atretic. During an analysis of follicle growth, we tested whether these 'Historical' criteria could correctly identify primary follicles that were not growing (and could thus be confirmed to be dying). In serial sections (n=4 ovaries), we counted not only the pyknotic granulosa cells in the largest section, but also granulosa mitotic figures and dying cells in each serial section of each primary follicle. If the number of mitotic granulosa cells in the entire follicle was greater than or equal to the number of pyknotic nuclei, we could infer that the total granulosa population was growing, and thus the follicle was growing. To our surprise, the historical criteria performed very poorly, with data summarized as follows. A single pyknotic nucleus in the center section corresponded to a 50% false positive rate where mitotic figures showed that the follicles were instead growing. Further, the historical criteria did not identify 6.3 follicles per ovary that mitotic scoring showed were not growing (false negatives). Evaluation of mitotic and pyknotic nuclei resulted in an improved method that allows the rapid, correct, and prospective identification of dying (and not growing) follicles with 98% accuracy by evaluating most often one follicle section, and at most two serial follicle sections. This improved, non-arbitrary method will greatly improve our ability to estimate the intact population of follicles in the ovary.

Age, Body Mass Index, and Number of Previous Trials: Are They Prognosticators of Intrauterine Insemination for Infertility Treatment?

[1]Isa, A.M.; [1]Abu-Rafea, B.; [1]Alasiri, S.A.; [2]Binsaleh, S.; [1]Ismail, K.H.; and [3]Vilos, G.A.
[1]Department of Obstetrics and Gynecology, Assisted Conception Unit, College of Medicine, King Saud University, Riyadh, Saudi Arabia
[2]Department of Surgery, Division of Urology, College of Medicine, King Saud University, Riyadh, Saudi Arabia
[3]Western Ontario University, London, Ontario, Canada

Objective: To examine whether pregnancy rate (PR) of intrauterine insemination (IUI) is related to certain demographic factors, such as age and body mass index (BMI), along with number of IUI cycles performed, in a set of infertile Saudi women.

Design: Prospective study in a public assisted conception unit.

Materials and Methods: During this prospective study (a 24-month period), 301 Saudi women with infertility underwent IUI in our infertility clinic. We investigated whether PR is correlated with patient age and BMI, and the number of IUI trials, in order to determine if they could be used as prognosticators of pregnancy success.

Results: The highest PR was 14.89% for ages 19-25 and the lowest PR was 4.16% for ages 41-45, indicating no statistically significant difference among PR in all age groups (p value of 0.225). Also, in terms of BMI, the highest PR was 13.04% for BMI ≥35 and the lowest was 7.84% for BMI of <25 to 18.5, indicating no significant difference among different BMI groups (p value of 0.788). One-cycle treatment, as expected, was more successful (PR=12.84%) than 2-cycle treatment (PR=5.75%), however, 3-5–cycle treatment still showed encouraging results (PR=17.24%); but the difference did not reach statistical significance (p value=0.167).

Conclusion: PR after IUI treatment remained approximately 10% from 19 to 40 years of age and declined after 40. Although no significant difference was observed among different age groups, earlier treatment is still recommended. There was a positive but not statistically significant correlation between PR and patient's BMI, indicating that BMI is not a determining factor. There was also no correlation between PR and number of IUI trials. Patients can thus try as many times as they want before moving on to in vitro fertilization (IVF) treatment.

Disclosures: Nothing to disclose

Funding: None

Compared to Blastomere Biopsy, Trophectoderm Biopsy Decreases Pregnancy Outcome and Is Associated with Early Embryonic Loss

Beyhan, Z.; Zody, R.; Hart, J.; and Keskintepe, L.
Sher Institute for Reproductive Medicine, Las Vegas, Nevada

Objective: To compare pregnancy outcome of euploid embryos in respect to type of biopsy (blastomere vs. trophectoderm) and transfer (fresh vs. frozen).

Design: Retrospective analysis in a multi-center private clinic setting

Materials and methods: A total number of 220 patients with a mean age of 37±0.5 were included in the analysis. All patients were selected to undergo aCGH-based genetic screening following laser-assisted biopsies performed either on Day 3 (BBx, 136 patients) or on Day 5/6 (TEx, 84 patients). 1508 embryos were biopsied and screened by aCGH in 270 IVF cycles. The study groups were comprised of (A) fresh embryo transfer (ET) after BBx, (B) frozen ET after BBx, and (C) frozen ET after TEx. All patients received either a single or double euploid blastocysts in their respective stimulation cycle (100 patients) or in subsequent frozen embryo transfer cycle (120 patients). Mean numbers of embryos transferred per cycle were 1.6. Vitrification was used for blastocysts cryopreservation.

The primary outcome measures of the study were clinical pregnancy rate, ongoing pregnancy and miscarriage rate. Fisher's exact test was employed for statistical analyses with a significance level of $p<0.05$.

Results: Of the 1508 embryos tested, 504 (33.4%) were euploid. Clinical pregnancy rates for the groups A, B, and C were 57%, 66% and 47%, respectively, and did not indicate statistical significance ($p=0.07$). A substantial number of embryos were lost in the group C, resulting in ongoing pregnancy rates of 49%, 58% and 31%, respectively. The difference in ongoing pregnancy rates was statistically significant when FET cycles were compared in regard to type of biopsy ($p=0.001$).

Conclusions: When transferred at the blastocyst stage, blastomere-biopsied cryopreserved embryos result in comparable pregnancy outcomes to those of transferred fresh, as opposed to some published reports, trophectoderm biopsy in this study seems to affect pregnancy outcome with a significant increase in the rate of early embryonic loss. Although not widely reported in the literature, the increase in the embryonic loss following trophectoderm biopsy may represent an intrinsic limitation of the procedure in older patient population by compromising the ability of an embryo to implant properly. Alternatively, this could reflect other technical and logistical challenges. More studies need to be performed to assess the effect of trophectoderm biopsy on the embryo's physiological status and pregnancy outcomes.

Disclosures: None

Funding: None

Gender Ratio Skew with Blastocysts Vitrified on Either Day 5 or Day 6

Volentine, K.; Keskintepe, L.; and Emmi, A.
Sher Institute for Reproductive Medicine—Central Illinois

Objective: To link gender ratio twist with blastocysts vitrified on either Day 5 or Day 6.

Design: Retrospective analysis in a private clinic set up.

Materials and Methods: Frozen embryo transfer (FET) data from April 2011 to December 2013 were collected and analyzed. Of the 150 FET procedures, 100 were with blastocysts vitrified exclusively on Day 5 (group 1), and 50 were with blastocysts vitrified exclusively on Day 6 (group 2). These two groups were compared using the Fisher's exact test based on age, number transferred, live birth rate, miscarriage rate, twin rate, and gender ratio. Statistical significance was considered $p<0.05$.

Results: The group 1 mean age was 32 years, transferring 1.4 blastocysts, resulting in 34% live birth. The spontaneous abortion (SAB) rate was 17%, and multiple gestations accounted for 2% of pregnancies. These findings did not differ from Group 2, who had mean age of 31 years, transferring 1.5 blastocysts, resulting in 24% live births. The SAB rate was 17%, and twin gestations accounted for 4% of pregnancies. On the other hand, the gender ratio was statistically different between groups 1 and 2, favoring more XY (52%) for Day 5 vitrified blastocysts than Day 6 (20%), $p=0.046$.

Conclusions: Although FETs utilizing blastocysts vitrified only on Day 5 or Day 6 have similar outcomes, statistically more XY babies were delivered with blastocysts vitrified Day 5 than Day 6. Data demonstrate that further studies are required to clarify the sex ratio skew, which was favoring females with Day 6 vitrified blastocysts.

Disclosures: None

Funding: None

Improving Pregnancy Rates in Women Over 40 Using Defined Laboratory Procedures

Wu, B.; Lu, S.; Silva, M.; and Gelety, T.J.
Arizona Center for Reproductive Endocrinology and Infertility
5190 E Farness Dr. #114 Tucson, Arizona, United States

Objective: Women over 40 years old have decreased chances for pregnancy even though current assisted reproductive technology (ART) has been successfully used to treat age-associated infertile couples. Current IVF laboratory improvements may increase the opportunity for pregnancy in older women.

Design: Prospective control study.

Materials and Methods: Women over 40 years old undergoing IVF/ICSI in our center in 2013 were used for this study. Laboratory methods were improved as follows: 1) two kinds of media were used for embryo culture. Patient's embryos with more than two fertilized eggs were cultured in Global and P1 medium, respectively; 2) embryo assisted hatching (AZH), all Day 3 embryos in women over 40 years old underwent assisted hatching by dissolving a hole on zona pellucida with Tyrode's solution and 3) the embryos were transferred in embryo glue medium (Vitro-life). 40-year-old women served as controls.

Results: A total of 42 women were used for this study. 40-year-old control women without AZH and embryo glue medium for transfer had a pregnancy rate of 29% (2/7). 41-42-year-old women could reach a 64% pregnant rate (14/22). However, women over 43 did not have a significant improvement in pregnancy rates, although one 45-year-old patient delivered a healthy baby.

Conclusion: Although women over 40 years old have a decreased chance for pregnancy, current laboratory procedures and improvements may significantly increase pregnancy rates in women aged 41-42.

Disclosures: Nothing to disclose

Funding: None

Limitations and Controversies in Determining the Predictive Value of Oocyte and Embryo Morphology Criteria: The Punctual Embryo Assessment Under Light Microscopy in a Time-Lapse Era

Figueira, R.C.S.; Azevedo, M.C.; and Borges Jr., E.
Fertility – Assisted Fertilization Center, Sao Paulo, Brazil
Sapientiae Educational and Research Institute in Assisted Reproduction, Sao Paulo, Brazil

Objective: To determine the predictive value of morphological criteria assessed punctually under light microscopy on blastocyst achievement and implantation potential.

Design: Transversal study in a private assisted reproductive technology program.

Material and Methods: This study consists of 743 IVF from 583 infertile patients submitted to assisted reproduction treatment from July 2011 to June 2014. A total of 5850 embryos individually cultured were evaluated regarding oocyte dysmorphisms independently identified, pronuclear score including: (i) number and size of pronuclei, (ii) number of nucleolar precursor bodies (NPB) and (iii) NPB distribution in each pronucleus and morphological score on Day 2 and 3 including: (iv) number of blastomeres, (v) blastomeres uniformity, (vi) fragmentation degree, (vii) presence of multinucleated blastomeres and (viii) cleavage speed. Associations between these variables and the probability of the achievement of a blastocyst or of an ongoing implantation were checked and candidate predictors were considered in order to construct a multivariable prediction model to rank embryos according to their blastocyst formation and implantation potential. Individual cycle, couple and treatments characteristics were also included into the model.

Results: Based on the bivariate analysis 43 potential predictive factors for the achievement of blastocyst on Day 5 and 20 potential predictive factors for blastocyst implantation potential were selected for the establishment of a prediction model using binary multiple logistic regression. The final prediction model included 14 independent predictive factors for blastocyst formation model and 4 independent predictive factors for blastocyst implantation potential. Early morphology criteria evaluated on Days 1, 2 and 3 provided additional selection power indicating blastocyst formation potential and the right blastocyst to transfer.

Conclusions: Despite recent advances in the reproductive medicine, based on the OMICs and time-lapse technology, in most IVF laboratories embryo selection for transfer is still based on morphological parameters evaluated under light microscopy. However, the subjectivity of morphological evaluation, as well as the wide diversity of embryo classification systems used by different fertilization centers implies in contrasting results, making the implementation of a consensus regarding different morphological criteria and their predictive value a difficult task. This model provides a helpful tool for clinics and embryologists in order to establish the best time for embryo transfer. Moreover, embryo selection optimization considering morphological criteria from the early embryo development represents a large potential to increase treatment success rates while minimizing the chances of multiple pregnancy.

Disclosures: Nothing to disclose

Funding: None

Results of Testicular Fine Needle Aspiration, Following Oocyte Retrieval Performed by the Same Physician

Shuman, J.; Simckes, E.; and Ray, L.
The Fertility Partnership of Saint Peters, Missouri

Objective: To summarize the past two years of our experience with testicular fine needle aspiration (TEFNA) for sperm recovery in obstructive and non-obstructive azoospermia in-vitro fertilization (IVF) cases.

Design: Preliminary, retrospective chart review of male patients undergoing IVF suffering from obstructive and non-obstructive azoospermia, operated on inside our clinic from January 2013 to December 2014.

Materials and Methods: Men were chosen for TEFNA based on one or more previous semen analysis showing azoopsermia. 24 men underwent TEFNA on the day of their female partner's scheduled oocyte retrieval. Following the last oocyte retrieval of the day, the same physician would then perform TEFNA using a handheld core biopsy system (Bard Magnum Biopsy) with an 18 gauge, 13 cm disposable needle. The physician would biopsy a core of semniferous tubule through a 2-3 mm incision made on the scrotum. The sample was then recovered and processed by the embryologist. Once sperm was confirmed, the physician would provide the patient with an ice pack for pressure to aid the recovery process. The sample was then processed for intra-cytoplasmic sperm injection according to the laboratory's TEFNA preparation protocol.

Results: Of the 24 men undergoing TEFNA, all 24 had identifiable mature sperm post TEFNA from one, or maximum 2 punctures per case. No complications arose as a result of surgery in any of the 24 cases. ICSI was performed in all 24 cases. A total of 285 metaphase II oocytes were retrieved with an average age of 33.62±3.7. All 285 were injected with sperm recovered from TEFNA, resulting in 184 (64.6%) normally fertilized zygotes. 17 of the 24 cycles underwent subsequent embryo transfer, resulting in 12 clinical pregnancies (70%). 4 of the pregnancies resulted in a spontaneous abortion, resulting in an ongoing pregnancy or live birth rate of 47% (8/17) per transfer.

Conclusions: Upon preliminary review of collected data, in house performance of TEFNA by the infertility clinic physician following oocyte retrieval is time saving, cost effective and efficient. Patients tolerated the procedure well and no side effects have been reported by the patients. The procedure circumvents the need to freeze sperm prior to oocyte retrieval in scenarios where a trained urologist is not available to perform a fresh TEFNA the day of oocyte retrieval. Use of TEFNA in the IVF clinic, performed by the infertility physician yields an acceptable fertilization rate, clinical pregnancy and ongoing or live birth rate per transfer, but is not a replacement for professional evaluation of a male with azoopsermia.

Disclosures: Nothing to disclose

Funding: None.

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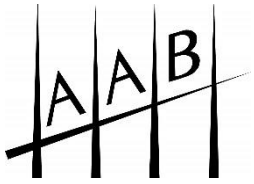

American Association of Bioanalysts
College of Reproductive Biology

906 Olive Street – Suite 1200
Saint Louis, Missouri 63101-1448
Telephone: (314)241-1445
Fax: (314)241-1449
Email: aab@aab.org • crb-aab@aab.org
Website: www.aab.org