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Abstracts

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ABSTRACTS – ORAL

ABSTRACTS – POSTER

24 Chromosome Aneuploidy Screening and FET Allows For High Pregnancy Rates and the Opportunity for Elective Single Embryo Transfer

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Objective: We report a non-selected series of patients that desired to use their own oocytes at IVF despite many of them having decreased ovarian reserve as defined by an AMH of less than 1.5. Blastocysts culture allows for developmental selection of the embryo cohort and 24 chromosome aneuploidy screening further defines those embryos capable of becoming a healthy live born.

Design: Retrospective Analysis

Methods: Retrospective case series from 10/1/2010 through 1/1/2014, where clinical pregnancy rate per ET, implantation rate, average number of embryos transferred, percent single embryo transfer (SET), SET clinical pregnancy rate, and percent of patients with poor prognosis (AMH <1.5) were analyzed for patient outcome data. These data include all patients that desired to use their own oocytes at IVF who underwent ICSI, sequential blast culture, laser trophectoderm biopsy, vitrification, and subsequent FET. SNP analysis was performed by Natera (San Carlos, CA).

Results:

Conceptions Reproductive Associates - Littleton, CO 10/1/2010 – 1/1/2014					
	Patient Age				
	<35	**35-37**	**38-40**	**41-42**	**43-44**
Number of transfers (total = 396)	156	106	86	31	17
Percentage of transfers resulting in pregnancies (FHT)	74.4%	77.4%	61.6%	71.0%	82.4%
Percent with single embryo transfer	65.4%	72.6%	76.7%	77.4%	88.2%
Implantation Rate	70.1%	74.1%	58.1%	71.1%	78.9%
Average number of embryos transferred	1.35	1.27	1.22	1.23	1.12
Percentage of patients with AMH < 1.5	27.1%	39.4%	37.7%	68.0%	80.0%
Percent aneuploidy (Patients with ET)	21.3%	29.0%	38.4%	42.0%	34.3%

Conclusion: Performing cycles utilizing the platform of 24 chromosome aneuploidy screening with vitrification allows patients the opportunity to obtain embryos with high reproductive potential while ensuring endometrial synchrony. A percentage of embryos were aneuploidy, particularly in women of advanced reproductive age and/or possessing decreased ovarian reserve. Identifying these embryos before transfer allows for the elimination of the number one cause of failed IVF, miscarriage and abnormal amniocentesis. We report a non-selected series of women, many of whom had an AMH value less than 1.5, who achieved a viable pregnancy utilizing elective single embryo transfer.

Disclosures: Nothing to disclose.

Funding: None.

Blastocyst Cell Differentiation and Phi: Assessment of Inner Cell Mass and Trophectoderm Cell Number in Mouse Embryos Cultured in Different Media Quality

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Introduction: The ability to observe and study early embryonic growth and development *in vitro* is beneficial in understanding similar events *in vivo*. For example, such studies permit us to insure optimal culture conditions which support blastocyst development and to maximize implantation potential. Many factors affect mouse embryo culture; one of the primary elements is culture medium. Embryo quality can be a combined subjective/objective assessment of percent blastocyst formation; total blastocyst cell number; or percent fragmentation. These assessments have been correlated with implantation potential. We have previously reported that an algorithm based on sperm head length-to-width ratios closely follows phi, also known as the Golden Mean (1.618), where morphologically abnormal sperm cells fall on either side of phi (Roudebush et al., 2006; Craig et al., 2013). Other areas where phi has been found include the symmetry of the Parthenon and the symmetry of the DNA molecule. DNA measures 34Å $\times$ 21Å for each full cycle of its double helix resulting in a phi of 1.619.To apply this concept to embryos and to further delineate the importance of an algorithm based on inner cell mass and trophectoderm cell numbers, we adapted the phi algorithm for use on mouse blastocysts that had been cultured in different media known to either optimally or sub-optimally support development.

Objective: To determine inner cell mass (ICM) and trophectoderm (TE) cell numbers in mouse embryos cultured in two different media known to produce varying embryo quality at the blastocyst stage and adapt the phi algorithm.

Methods: One-cell mouse (CFW strain) embryos were cultured in Ham's F10 (HF10) or Minimum Essential Medium-alpha modification (αMEM) to the expanded blastocyst stage. Cell numbers for ICM and TE were determined by differential labeling with polynucleotide-specific fluorochromes (Hoeschst 33258 and propidium iodide). Phi (ϕ) for each blastocyst was calculated as follows: ϕ = ICM+TE)/TE. Data were analyzed by the *t* test.

Results: A total of 691 mouse blastocysts were analyzed as described above and results are presented in the table below.

Medium	n	% Development	ICM (mean)	TE (mean)	ϕ (mean)
HF10	324	56	33.6	67.5	1.489
αMEM	367	87	58.8	92.8	1.634
P		<0.001	<0.05	<0.05	<0.05

Conclusion: In the present study, calculating for phi proved to be an effective way to help determine overall mouse embryo quality following culture. Additionally, culture medium formulation demonstrated a significant role in determining overall blastocyst quality as illustrated by phi in the mouse model. Further studies are required to demonstrate the importance of phi and its potential relationship to embryonic implantation.

Blastocyst Quality, Phi and Implantation Potential: An Objective Analysis for General Classification and Blastocyst Selection for Single Embryo Transfer

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Introduction: Embryo grading is a mixture of subjective and objective assessments (e.g. cell stage; cell number; degree of fragmentation) that has been minimally linked with implantation potential. We have previously found that sperm head length-to-width ratios closely follow phi (1.618; http://www.goldennumber.net/what-is-phi/), where morphologically abnormal sperm cells fall on either side of the mean of morphological normal sperm cells (Roudebush et al., 2006; Craig et al., 2013). To apply this result and to further delineate the importance of length-to-width ratios, we updated the algorithm for use on human blastocyst embryos classified as being of high quality and reported to have either successfully implanted or failed to implant.

Objective: To determine length-to-width ratios in high quality human blastocysts following embryo transfer.

Design: Retrospective post-transfer analysis of length-to-width ratios in human blastocysts calculated for phi (length-to-width ratios) graded as high quality at the time of embryo transfer.

Methods: Human blastocyst stage embryos that were graded as being of high quality at time of embryo transfer (Veeck and Zaninovic, 2003) were measured for length and width along the inner cell mass axis. Length-to-width ratios (phi; ϕ) were calculated (ϕ=[L*W]/L) for each human blastocyst and assigned to the resulting phi category: <1.537 (<95% of phi; ϕ_{95}); B=1.537-1.699 (phi $\pm$ 5%; $\phi_{95\text{-}105}$); or >1.699 (>105% of phi; ϕ_{105}). Data were analyzed by analysis of variance.

Results: Pregnancy rates for the three different phi groups are presented in the following table:

Phi Group	Pregnancy Rate (%)
ϕ_{95}: <1.537	37.5
$\phi_{95\text{-}105}$: 1.537-1.699	100
ϕ_{105}: >1.699	33.3

There was a significant difference (P<0.001) in pregnancy rates between the three different phi groups. All pairwise multiple comparisons (Tukey test) were also found to be significantly different (P<0.05).

Conclusion: Human blastocyst stage embryos measured for length and width provide a more objective assessment of morphology and may prove to be more uniform, repeatable and reliable than standard subjective assessments. Selecting human blastocysts with a calculated phi ratio that falls within 5% of phi may result in improving embryo transfer success. Further studies are required to demonstrate the importance of phi and the prediction of implantation potential for single embryo transfers.

Comparison of a Sequential Culture Media and Single Step Culture Media Systems

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Objective: The objective of these studies was to compare a sequential culture media system and a single culture medium in a clinical IVF laboratory setting.

Design: The design of this media evaluation was a prospective randomized trial. A T-test for independent samples was employed to evaluate the utilizable blastocyst formation data.

Materials and Methods: In study 1: Culture system A consisted of a sequential three step media system with a fertilization medium utilized on D0 of culture, a cleavage stage medium utilized from Day 1 to Day 3 of culture and a blastocyst medium utilized from D3 to D6 of culture. Culture system B consisted of a single culture medium. All patients in Study Period 1 (n=129) had oocytes randomly divided between the two different culture systems. Culture in the single culture medium was carried out in the same manner as the sequential system with embryos moved into fresh medium on D1 and D3. In Study Period 2, all oocytes from retrieval patients (n=133) were randomly divided between two different single step culture media (System B and System C). Embryos were cultured in four well plates with 500µL of medium with a 500µL oil overlay. Cultures were continuous with no medium change or replenishment throughout the culture period. Utilizable blastocysts consisted of blastocysts that were transferred and/or frozen and were expressed as a percentage of two-PN embryos.

Results: In Study Period 1, 641 two-PN embryos were cultured until day 6 in Medium A and 627 two-PN embryos were cultured in Medium B. Utilizable blastocyst development was 40.98% in medium A 42.55% in medium B (NS). In study 2 574 2-PN embryos were cultured in Medium B and 590 2-PN embryos were cultured in Medium C. Utilizable blastocyst development in Medium B was 49% and Medium C was 51% and (NS).

Conclusions: A single culture medium can produce utilizable blastocysts at the same rate as a sequential culture media. Two commercially available single culture systems evaluated produce utilizable blastocysts at the same rate. Utilization of a single culture medium can simplify the culture process. This in turn can lead to risk reduction and cost savings for the laboratory.

Disclosures: None.

Funding: None.

Effect of Denuding Oocytes Following Brief Co-Incubation with Sperm for Fertilization Assessment with Time-Lapse Imaging

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Background: Time-lapse imaging used in conjunction with embryo culture is becoming more widespread in state-of-the-art In Vitro Fertilization (IVF) laboratories. This technology allows assessment of embryo development without removing embryos from the culture environment or even requiring a technologist be present in the lab. Images are compiled and stored in a remotely accessed computer where image video can be reviewed and annotated for key milestones in embryo development. This imaging requires oocytes be free of coronal cells to allow for an unobstructed view of the cytoplasm before placement into the EmbryoScope™. Previous studies have shown that brief co-incubation for IVF is associated with increased ongoing and clinical pregnancy rates (Huang, et al, 2013). Following this co-incubation period coronal cells around the oocyte are normally left undisturbed. However, when using the EmbryoScope™ these cells must be removed.

Objective: To determine if fertilization rates are affected between complete denuding (D) versus not denuding (ND) oocytes following a 3-hour co-incubation with sperm for conventional insemination patients.

Methods: Up to 5 oocytes were placed into the center well of an organ culture dish containing 1.0ml of Global Fertilization™ media supplemented with 5mg/ml of Human Serum Albumin (HSA) and covered with 1.0ml of Global Lite Oil™. Four hours following the oocyte retrieval, between 200,000 and 350,000 motile sperm were added to each dish. Following a 3-hour co-incubation period, half of the oocytes were randomized to be completely denuded and placed into a 20 microliter drop of Global™ media supplemented with 10% SPS and the other half were carefully moved to a fresh media drop without disturbing coronal cells. Between 16 and 18 hours later, fertilization was assessed based on presence or absence of pronuclei.

Results: A total of 136 oocytes from 8 patients were assessed for fertilization following randomization between complete denuding (69) or no denuding (67) following a 3-hour sperm/oocyte co-incubation period. Following in Table 1 are results for 2PN, 1PN and ≥3PN fertilization.

Table 1

Treatment	2PN (%)	1PN (%)	≥3PN (%)
Complete Denuding	46/69 66.7%	5/69 7.2%	5/69 7.2%
No Denuding	43/67 64.2%	8/67 11.9%	4/67 6.0%

Conclusions: This data set shows no significant difference in normal or abnormal fertilization rates between complete denuding versus no denuding of oocytes following 3-hour co-incubation for IVF. This suggests it is safe to denude oocytes for placement into the EmbryoScope™ for fertilization assessment and extended culture. Further studies are underway to compare embryo development between these two groups.

Disclosures: None.

Funding: None.

Liquid Nitrogen Vapor Sealing of Straw Containers can be Unsafe and Detrimental to Embryo Survival: A microSecure™ Re-Vitrification (rVTF) Model

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Objective: Aseptic closed vitrification (VTF) systems that create an in situ vapor-phase storage environment around the device (e.g., µS-VTF, HSV) in liquid nitrogen (LN_2) have proven to be clinically effective for blastocyst (BL) VTF. Both µS-VTF and HSV are considered safe and secure systems because they are loaded and weld-sealed under ambient (20-22°C) conditions. At least one traditional open VTF system (Cryotop) is now modifying its storage procedure in an attempt to gain FDA approval, requiring sealed containers. The goals of our study were to show that: 1) ambient loading/ sealing of flexipettes (µS-VTF; Control) for VTF was equally effective to ultra-rapid cooling and LN_2 vapor loading/sealing (UR-TRT); and 2) LN_2 vapor sealing of straws is an unsafe, unreliable and potentially harmful practice.

Design: Using a rVTF model on research consented, discard embryos, 41 BL were randomly assigned to either Control (n=19) or UR-TRT (n=22) rVTF.

Materials and Methods: Standard "microSecure vitrification warming" (see YouTube video) of flexipettes (Cook, 300µmID) was first performed without extraction/elution of BL. After 10sec warming in 37°C in 0.5M Sucrose solution, the flexipettes were dried with sterile gauze and directly re-vitrifed at 1min post-warming, by either: 1) Control µS-VTF; or 2) UR-TRT where flexipettes were dipped in LN_2 (5sec) and inserted into open-ended straws held in LN_2, with the open end held in vapor phase (3cm) and hand-sealed closed for storage. Subsequently, warming of µS-VTF Control and UR-TRT straws was repeated followed by standard elution in sucrose solutions and isotonic equilibration before BL culture. Survival was assessed at 0hr and 24hr. Differences in BL survival and formation (BL_{0hr} survival÷BL_{24hr} reformation) were assessed by a χ^2 test (*$p<0.05$).

Results:

Observations	µS-VTF Control	Ultra-Rapid TRT	No Differences
# BL rVTF/warmed	19	19$^{\Psi}$	N.S.
# Survived (0hr)	15 (78.9%)	16 (84.2%)	P=0.67
# Survived (+24hr)	13 (68.4%)	10 (52.6%)	P=0.32
BL Reformation (+24hr)	13/15 (86.67%)	10/16 (62.5%)	P=0.12

$^{\Psi}$ 3 UR-TRT straws exploded before procedure modification (15 sec N_2 outgassing).

Discussion: Some decline in BL survival post-rVTF was expected. However, this study has revealed that UR–closed VTF may be a potentially unreliable, unsafe and less effective procedure. The inability to guarantee complete seals of super-cooled straws or the possible entry of N_2 vapors inside a straw upon sealing create significant risks which are unnecessary quality control variables absent in standard aseptic, closed VTF methods.

Disclosure: This study was conducted by 2 dedicated summer-student interns.

Funding: None.

Accurate Diagnosis as a Prognostic Factor in Intrauterine Insemination (IUI) Treatment of Infertile Saudi Patients

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Objective: The study meant to define the prognostic factors that help in prescribing intrauterine insemination (IUI) for infertility treatment which remains an area of continuous improvements.

Design: We prospectively studied the diagnostic indications of the IUI-treated patients and their corresponding pregnancy rates (PR) in a Saudi group of patients for a period of 20 months.

Materials and Methods: We analyzed the indications of IUI cases that we conducted to those eligible patients- after signing the appropriate informed consent forms for the study- over a period of twenty months, and the PR that corresponded to each group.

Results: The highest PR, 18.87%, of the polycystic ovarian syndrome (PCOS)-only diagnosed patients, was significantly higher than the average PR of all other indications combined, 7.22%, (P= 0.011). The second highest PR, 14.0%, of the tubal factor (TF)-only indication, was double the PR average of all other indications combined, though it did not reach significance (P= 0.286). However, PCOS and TF accompanied by other indications caused the PR to drop to 5.88% and 5.56%, respectively. However, a group of some hormonal-imbalance based indications had the least PR (0.0% to 2.70%). Those indications were high FSH, hyperprolactinemia, hypogonadotrophy, hypothyroidism, and endometriosis. The rest of the indications had an average PR (8.33% to 11.11%).

Conclusions: Unless the cause of infertility was severe hormonal-imbalance based, there was a reasonable chance of conception after IUI treatment, especially if the indications were PCOS or TF.

Disclosures: The authors do not have any conflict of interest with any party whatsoever.

Funding: This study was sponsored by the College of Medicine research center in King Saud University, Saudi Arabia.

Cryopreservation of Testicular Sperm Prior to Vasovasostomy: A Ten-Year Experience

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Objective: It has been more than 20 years since reports of the first ICSI using sperm retrieved directly from the testicle. Not only has ICSI become standard procedure, but testicular sperm extraction (TESE) has been routinely used for males with obstructive and non-obstructive azospermia. The purpose of this study was to review testis biopsy specimens from vasectomized men during the period September, 2003 - January 2014.

Design: Retrospective review of testicular biopsy tissue submitted for cryopreservation before vasectomy reversal.

Materials and Methods: Testicular tissue was submitted for processing and storage prior to or at the time of vasectomy reversal. All specimens were placed in Ham's F-10 or normal saline and transported to the lab within 1-2 hours. Aliquots of the specimen before and after tissue maceration, were examined on a Makler chamber, and the numbers of sperm seen determined. Sperm were considered motile if there was progressive or non-progressive motion. Following addition of Ham's F-10 with 7.4% glycerol, the specimen was divided into sterile vials, frozen at -10 to -25C for 5 minutes, placed in liquid nitrogen vapor for a minimum of 30 minutes, then stored in the liquid phase. All specimens were processed by the same two technicians. All biopsies were done by the same physician. The data was expressed as the mean $\pm$ S.D.

Results: **A** total of 148 TESE specimens were submitted from men with a mean age of 43 years (1.4), (range of 29-59 years). The mean initial sperm count (million/ml) was 1.25 (0.78). 106 showed no motility. Of the 42 specimens with motility, the mean motility was 32% (5.66). For an additional 49 specimens (not included), half had no sperm on the entire grid, and half had sperm outside the grid. A post-thaw analysis was not done. Of the stored specimens, 62 were subsequently discarded, 38 requested release of 1 or more vials for use, 60 currently are storing vials and 6 have abandoned their stored vials. An additional 49 TESE specimens were received, no sperm were found, and were not included in the above data.

Conclusions: Adequate numbers of motile or non-motile sperm can be obtained from testicular tissue of vasectomized men for use in assisted reproduction. It is conceivable then, that TESE could be considered rather than surgical reversal of vasectomy. However, banking of TESE specimens before or during vasectomy reversal is an appropriate consideration to compensate if the vasectomy reversal is not successful.

Disclosures: None.

Funding: None.

Do Day 3 Embryo Transfer Patients Benefit from Extended Embryo Culture and Day 5 Embryo Transfer?

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Objective: To determine if Day 3 (D3) embryo transfer patients would benefit from extended embryo culture and Day 5 (D5) embryo transfer.

Design: A retrospective review between 9/1/10 to 12/20/13 of 947 cycles, <40 years of age, having had either a fresh D3 transfer, a fresh D5 transfer or a frozen D5 embryo transfer from blastocysts cryopreserved from a failed D3 transfer cycle. Clinical outcome of fresh D3 and fresh D5 transfers were compared. Patients with unsuccessful fresh D3 transfers having blastocyst(s) cryopreserved were identified and followed for clinical outcome as a result of subsequent vitrified-warmed blastocyst transfers.

Materials and Methods: Female patients were subjected to controlled ovarian hyperstimulation. Ovarian follicles were aspirated trans-vaginally by aspiration 34-36 hours post hCG injection using ultrasound guided needle. Follicular aspirates were searched for Cumulus Oocytes Complexes (COCs) under stereomicroscope. Embryos created after IVF and ICSI were cultured under standard conditions (Cleavage and blastocyst media with protein supplementation under 6% CO_2, 5% O_2 at 37ºC).

Fresh embryo transfers were performed either on D3 or D5 depending on patient age, total embryos in culture and number of good quality cleavage stage embryos. Supernumary embryos were cryopreserved by vitrification only at blastocyst stage.

Vitrified-warmed blastocysts were transferred after preparing the uterus in subsequent treatment cycles.

Results: Clinical pregnancy rate (fetal heart beat) after fresh Day 3 embryo transfer was 43.3%, while the clinical pregnancy rate for fresh Day 5 embryo transfer was 59.9%. Of the 301 Day 3 transfer patients whom did not achieve a pregnancy, 37.6% had good quality excess embryos frozen at the blastocyst stage. Ten of these patients came back for a FET cycle, with a 60.0% clinical pregnancy rate. Clinical pregnancy rate (fetal heart rate) after fresh D5 embryo transfer (59.9%) was significantly higher compared to that of fresh D3 embryo transfer (43.3%) ($p<0.001$).

Conclusion: Fresh Day 3 embryo transfer patients with negative outcomes and excess embryo(s) frozen at blastocyst stage may have benefitted from extended culture and selection of embryos for transfer at the blastocyst stage.

Disclosures: None.

Funding: None.

Effect of Accidental Warming Intervals on the Survival of Vitrified Human Blastocysts

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Objective: Current dogma suggests that the viability of vitrified blastocysts (BL) can be compromised by a brief exposure to room temperature. We propose that this critical sensitivity is likely to be vitrification (VTF) device/solution dependent. Using an aseptic, closed VTF device, microSecure (µS-VTF), we hypothesize that a double container, in situ vapor-phase insulation provides extra security to vitrified embryos exposed to warming and re-VTF (rVTF). Our experimental aim is to determine the theoretical threshold of survival loss for accidentally warmed µS-VTF-BLs.

Design: After some preliminary investigation, 2 accidental thaw intervals were selected. Using an apriori arrangement of 3 Test time treatments (0 sec, 10 sec and 1 min), 30 BLs vitrified in non-DMSO solutions (I.C.E.) were warmed to mimic a time of accidental exposure at ambient conditions (21-22°C). Our Positive/Negative Control and Test groups each consisted of 10 research consented Day 5 grade "A" trophectoderm µS-VTF-BLs.

Materials and Methods: The Test and Negative warming/rVTF treatments were conducted. After standard, rapid µS-VTF warming (6000°C), BL were eluted in T1-T4 sucrose solutions at 3min intervals, and equilibrated in H-LG medium for 5 min (37°C). All BLs were incubated in a low O_2, humidifed environment at 37°C in microdrops of LG medium + 7.5%SS under oil for 24 hrs. The BLs were assessed for continued development (BL expansion/hatching=survival). Primo Vision time lapse imaging was also used to determine if any difference in re-expansion of Control and Test BL was observed. Differences in BL survival were assessed by a χ^2 test ($p<0.05$).

Results: No differences in rVTF survival were detected.

Treatment Interval:	Positive Control 0 sec	Test 10 sec	Negative Control 1min
# Thawed	10	10	10
0hr # Survived (%)	10 (100%)	10 (100%)	9 (90%)
24hr # Survived (%)	10 (100%)	10 (100%)	8 (80%)

Conclusion: We have shown that BL vitrified by µS-VTF in non-DMSO solutions are more resilient to sub-optimal warming/devitrification than previously considered. Normal survival, based on cellular integrity and re-expansion, did occur at 1 min but may be approaching a detrimental threshold as some 24 hr embryo degeneration did occur. Phase II of the study will involve evaluation of additional VTF systems (devices/ DMSO/EG solutions) to validate our hypothesis that aseptic, closed devices provide extra security from accidental warming of VTF-BLs.

Disclosure: The authors have no commercial interests in the µS-VTF product.

Funding: None.

Effect of Incubation Times Before and After the Removal of Cumulus Cells on the Developmental Competence of Oocytes in Patients Undergoing Intracytoplasmic Sperm Injection (ICSI) Treatment Cycle

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Objective: To determine if the incubation times before and after the removal of Cumulus Complexes affect the developmental potential of oocytes.

Design: Retrospectively, fertilization rates and developmental patterns of embryos created after sperm injection of oocytes held three hours prior to and one hour after cumulus removal will be compared to those held two hours prior to and two hours after cumulus removal.

Materials and Methods: Female patients were subjected to controlled ovarian hyperstimulation. Ovarian follicles were aspirated transvaginally 34-36 hours post hCG injection using an ultrasound guided needle.

Follicular aspirates were searched for Cumulus Oocyte Complexes (COCs) under stereomicroscope. COCs retrieved for ICSI during Block 71 (4/20/13 through 7/26/13) were held in Quinn's Advantage® Cleavage Media and those retrieved in Block 72 (8/17/13 through 12/13/13) were held in Quinn's Advantage® Fertilization Media with 10% serum protein substitute (SPS) under standard culture conditions (6% CO_2, 5% O_2 at 37ºC) for three and two hours, respectively. COCs were denuded in hyaluronidase and held in Quinn's Advantage® Cleavage Media under standard culture conditions for one hour during block 71 and two hours during block 71, prior to sperm injection.

After ICSI, oocytes from both study groups were cultured in Quinn's Advantage® sequential culture system under standard conditions. Fertilization checks were performed between 16 and 19 hours post ICSI. Zygote/embryo development was checked at 25, 42-44, 66-68, 114-118, 140-142 hours post ICSI.

Results: Fertilization rates during block 71 and 72 were 70.2% and 68.8% respectively differing non-significantly ($P>0.5$). Percentage of top and high quality embryos were 35.6% during block 71 and 36.8% during block 72 without any significant difference ($P>0.5$). Conversion of injected oocytes to good quality blastocysts was also non-significantly different ($P>0.5$) between block 71 (19.8%) and block 72 (17.4%).

Conclusion: Data analyzed so far suggests that reducing the incubation interval between oocyte retrieval and enzymatic removal of cumulus cells to two hours and increasing the holding time of denuded oocytes before ICSI to two hours did not affect fertilization and blastocyst formation. The results obtained are from a relatively small data set. More data is being collected for further analysis.

Disclosures: No disclosures.

Funding: No funding.

Evaluation of *Helicobacter pylori* IgA Immunoassay: Concerns on High Rates of Positive IgA Results in IgG Negative Healthy Subjects

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Objective: Helicobacter *pylori* infects more than 50% of the world's population and is known to be associated with peptic ulcer disease and gastric carcinoma. IgA positive results are found in a subset of IgG negative patients, despite IgG being the most sensitive and specific H. pylori serology test. Measurement of IgA antibodies can therefore aid the diagnosis of these patients. In this study, we evaluated the only H. pylori IgA assay that has been cleared by the FDA for *In vitro* diagnosis (IVD).

Design: The validation study was designed following the Clinical Laboratory Standards Institute guidelines.

Materials and Methods: Residual patient serum samples and healthy donor samples were used in the evaluation. H. *pylori* IgA antibody levels were determined using QUANTA Lite™ *Helicobacter pylori* IgA kit (Inova Diagnostics, Inc.) following manufacturer's instructions. The imprecision was estimated using pooled negative and positive patient samples. Accuracy was assessed by comparison with a lab-developed test (LDT) performed at a large reference laboratory. Reference interval was verified using 120 IgG negative healthy subjects.

Results: The intra-assay and inter-assay coefficients of variation (CV) were 9.7-13.5% and 4.7-12.6% respectively. Qualitative agreement between the IVD assay and the reference laboratory LDT was 80%. Most of the discordant results presented as negative by the reference laboratory LDT but equivocal or border line positive by the IVD assay. Moreover, about 50% of the discordant samples were IgG positive. About twenty percent of IgA positive samples tested by both methods were negative by the IgG test. Sixteen percent of IgG negative healthy subjects tested positive using the IVD assay. The reference interval established using the population served by our laboratories was 33.1U, which is higher than that claimed by the manufacturer (25 U).

Conclusions: This H. *pylori* IgA antibody assay showed good precision and reasonable concordance with a LDT using in a large reference laboratory. However, it might be necessary to evaluate positive rates in the local healthy population and communicate with physicians to avoid triggering unnecessary expensive and invasive testing procedures.

Disclosures: Nothing to disclose.

Funding: None.

Incidental Exposure of Pronuclear and Cleavage Stage Embryos to Fluctuations in Oxygen Concentrations During Incubation: Continued Development at Extended Culture, Freezing and Pregnancy Outcomes

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Objectives: To evaluate the development and pregnancy outcome of embryos incidentally exposed to fluctuations in oxygen (O_2) concentrations at different cleavage stages. Fluctuations in concentrations were caused by excessive liquid nitrogen (LN_2) or nitrogen gas (N_2). Development to the blastocyst stage for transfer and freezing, and pregnancy outcomes were considered as the endpoints to assess the impact of fluctuations in O_2 concentrations.

Design: Case report.

Materials and Methods: Embryos from five IVF cycles were being incubated between Day 1-3 post-insemination. All embryos were in the same incubator (HERAcell 150) at 37˚C, 5.5% CO_2 and 5% O_2. The culture media consisted of cleavage media (Sage) supplemented with 10% (v/v) serum protein substitute (SPS; Sage). Embryos were cultured in microdrops (30 µl; 1-2 embryos per drop) of culture media covered with mineral oil. Embryos were then transferred to blastocyst media/SPS for extended culture (Days 3-6). Incubators were connected via a manifold to two CO_2 tanks (800 psi; primary and back-up), and via a single line to a large capacity LN_2 cylinder (160 liter). Also, N_2 tanks (800 psi) were available as back-ups.

Triggering of the remote alarm for the incubator and excessive LN_2 consumption was noticed early in the morning during routine embryo culture procedures. Three of the IVF cases had embryos on day 3, one case on day 2 and one case on day 1 of culture. The empty LN_2 cylinder was replaced with a back-up N_2 tank. The excessive N_2 consumption continued during the next 48 hrs, which required continued replacement of N_2 tanks until the situation was resolved (weekend issue). Continued embryo development was assessed by evaluation of expected stages on Day 3 (6-8 cells) and Day 5-6 (Morula-Blastocyst).

Results: Fluctuations in LN_2/N_2 consumption levels persisted for 48-60 hrs until the situation was normalized and resolved. Other environmental conditions inside the incubator were unaffected, including: % CO_2, temperature, humidity and pH of culture media kept as indicators. The range of O_2 fluctuations was between 10-17%, as measured post-triggering of the remote alarm system. The cause of the excess LN_2/N_2 consumption and fluctuation in O_2 levels was identified as a twisting of the tubing in the incubator water pan, which feeds information to the O_2 sensor that controls injection of N_2 into the incubator. Embryo assessment parameters during this incident are summarized in the Table below.

	# Initial Embryos	Day of Culture/ O_2 fluctuation	% Development Day 3	% Development Day 5	% Embryos Frozen	Ongoing Pregnancy
Case 1	10	3	100	90	30	Yes
Case 2	6	3	100	83	0	Yes
Case 3	5	3	40	60	0	Yes
Case 4	2	2	100	50	0	Yes
Case 5	6	1	100	100	0	No

Conclusions: A low O_2 concentration is considered necessary for optimal incubation conditions in human embryos. In this case, embryo development did not seem to have been compromised by fluctuations in O_2 levels. Also, the outcome of pregnancies for the five cycles was not compromised (80% ongoing pregnancy). However, full expansion of blastocysts as compared to embryos prior and after the incident may have been diminished, as well as, availability of embryos for cryopreservation. It is encouraging to know that embryos can continue developing without compromising pregnancy rates in an emergency situation as described in this report with fluctuation in O_2 levels, and as long as the other environmental conditions inside the incubator remain constant.

Disclosures: Nothing to disclose.

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Preparation of Warming Medium for Vitrified Specimens – Effects on the Final Warming Temperature

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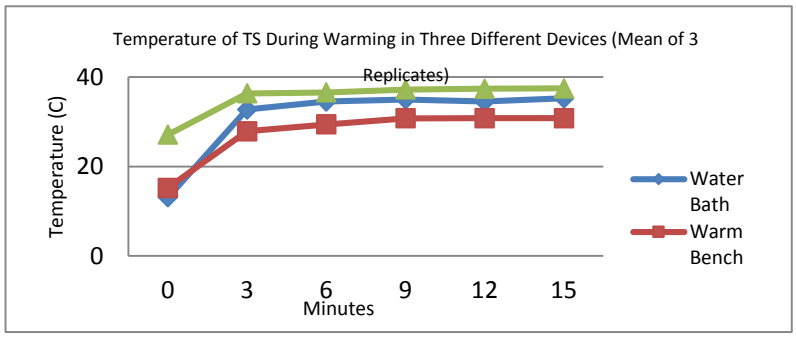
Objectives: One of the critical steps in vitrification is the warming velocity which moves the specimen from a glass state to a liquid and avoids recrystallization. In these experiments different methods of preparing warming media for warming vitrified embryos were examined to determine their effects on the actual warming temperature.

Design: In the first experiment, warming of media in a water bath was compared to a convection incubator and a warm bench top. In the second experiment, the effects of tube inversion and pipetting into either a warm or room temperature dish were examined.

Materials and Methods: 1.0 M Sucrose (TS) was placed into 4 ml snap cap tubes for warming. In the first experiment, temperatures were measured without inverting the tubes. For the second experiment, tubes were removed from the 37 C water bath and inverted 2 times and then pipetted with a 5 ml glass pipette into either a warmed or room temperature dish. All temperatures were measured with a T-type thermocouple (Testo 926 electronic thermometer).

Results: All 3 warming devices produced plateau temperatures within 6 minutes. At 6 min, temperatures were 36.3, 34.5 and 29.4 for the incubator, water bath and bench top, respectively. Both tube inversion and pipetting decreased the final temperature of the warming solution 3.5 C and 6.4 C (warmed dish and room temperature dish). In a prior experiment inversion alone decreased the temperature almost 1 C.



Conclusions: How warming solution is prepared can result in a drop from an initial temperature of 37°C to less than 30°C. Warming on a bench top, tube inversion and pipetting should be avoided during preparation of warming solutions. It is possible that these differences can result in significant changes in outcomes and may account for some differences in success of egg banking recipients.

Disclosures: None.

Funding: None.

Relationship of IVF/ET Cycle Volume and Outcomes in Texas for 2012: A Follow-up to Outcomes in 2011

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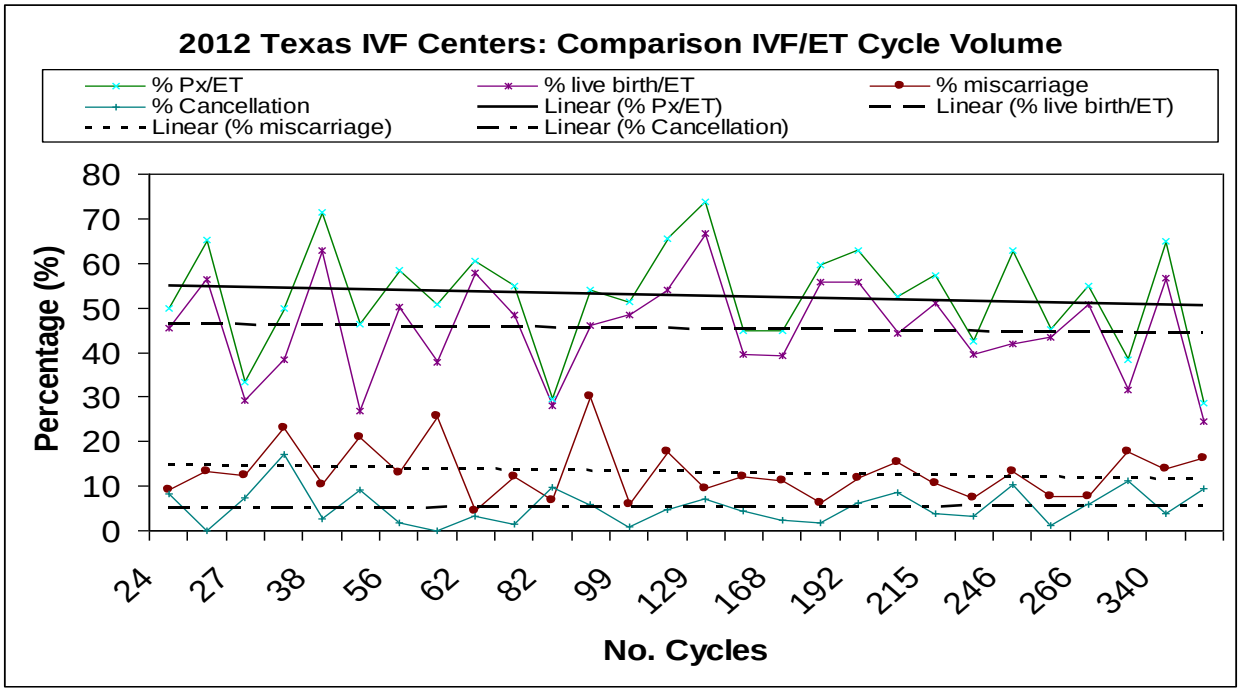
Objectives: We previously reported the observation of an inverse relationship between the volume of IVF cycles and pregnancy outcomes for clinics in the State of Texas for 2011. The possibility that quantity of cycles of IVF/ET performed by a center might influence outcome is the subject of this current analysis. Thus, we propose to analyze current data for the following reporting year (2012) and to determine if the previous data trends can be validated.

Design: Retrospective analysis of the relationship between the number of IVF cycles per clinic and pregnancy outcomes in the State of Texas for 2012.

Materials and Methods: Data provided in the Society for Assisted Reproduction Technology (SART) Clinic Report for 2012 was analyzed (patients 37 years of age and younger). Data analysis included clinics in Texas performing a minimum of 20 cycles upwards to a volume of 500 cycles per year. The relationship between the following variables (per embryo transfer) was tested by regression analysis: number of IVF cycles, % pregnancy, % live birth, % cycle cancellation and % miscarriage.

Results: Regression analysis showed an inverse trend between an increase in the number of IVF cycles and a decrease percentage for pregnancy and live birth per transfer, as well as, for the cancellation and miscarriage rates (See Table).



Conclusions: For the SART reporting year of 2011 in Texas, the volume of cycles performed by specific clinics was found to inversely correlate with the likelihood of pregnancy resulting in a live birth. A positive correlation was found for the number of cycles performed and the occurrence of miscarriage, and for the likelihood of cycle cancellation. The statistical analysis for all of those variables was found to be

significant in 2011. For the same comparisons in 2012, we found that the pregnancy and live birth rate are still inversely correlated, but the differences are not significant. The trend for the percentage of cancellations and miscarriage was inversely correlated with an increase in the number of cycles (non-significant differences), which represents the opposite of what we found in 2011.

Fluctuations in decisions involving elective single embryo transfer, embryo banking cycles and genetic screening cycles may have affected the outcomes for 2012. For future studies, we also propose to study the available data in the past 3-4 reporting years for the purpose of individual and pooled data comparisons.

Disclosures: Nothing to disclose.

Funding: None.

Rescue Intracytoplasmic Sperm Injection (ICSI) and Delayed Embryo Transfer (ET) Resulting in Live Birth: A Case Report

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Objective: To report a unique case of failed fertilization, rescue ICSI, delayed blastulation and implantation resulting in a live birth.

Design: Case report.

Materials and Methods: A 30yo G0P0 patient began a superovulation / intrauterine insemination (IUI) cycle with 150 units of follicle stimulating hormone injections started on cycle day 3. Monitoring was performed at an offsite physician's office. The cycle was converted from IUI to in vitro fertilization (IVF) on cycle day 13 when she had 12+ follicles and an estradiol (E2) of 1541 pg/mL. On cycle day 16, there were 22 follicles ≥ 10mm and E2 was 1965 pg/mL. She was given 10,000 IU human chorionic gonadotropin (hCG) on cycle day 17 with transvaginal oocyte retrieval occurring 35 hours later.

Results: Eighteen cumulus oocyte complexes were retrieved and conventional insemination was performed at 5.17 hours post retrieval (hpr). Fertilization check was performed at 17.75 hours post insemination (hpi) showing no fertilization. After consulting with the physicians and patient, rescue ICSI was performed on 15 metaphase 2 (MII) oocytes at 27.25 hpr. Fertilization check was repeated at 10 hours post rescue ICSI showing four 2 pronuclei and three 1 pronuclei embryos. Standard embryo culture technique was used. Six days post retrieval and five days post rescue ICSI, there were two remaining embryos, one early morula and one blastocyst. Embryo transfer occurred the next day (seven days post retrieval and six days post rescue ICSI) with the transfer of one early blastocyst (1BB) and one expanded blastocyst (4BA). Serum beta hCG was drawn 16 days post retrieval and measured 225 IU/L, increasing to 744 IU/L two days later. A singleton intrauterine pregnancy with fetal heart beat was observed at 6 weeks and 1 day gestation. A healthy baby boy weighing 2.83 kg was born at 38 weeks gestation.

Conclusions: Failed fertilization is a devastating outcome for physicians and patients. While rescue ICSI is a tool of last resort and not advisable in cases of failed fertilization post-ICSI, this study demonstrates that it can result in a live birth. To our knowledge, this is the first report of rescue ICSI with a 7 day post retrieval embryo transfer resulting in a live birth.

Disclosures: None.

Funding: None.

Semen Cryopreservation Prior to IVF: Analysis of 165 Patients During a Ten-Year Period of Time

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Objective: Males may freeze semen before IVF if there might be difficulty collecting on schedule, or ifhe might not be available for partner's cycle. The purpose of this study was to review the sperm bank database for men storing sperm prior to IVF from September, 2004 - January 2014.

Design: Retrospective review of sperm bank database.

Materials and Methods: A total of 133 patients, stored 165 specimens. All specimens were collected by masturbation following 1 to 14 days abstinence. For unwashed semen, an equal volume of TEST-yolk buffer with 15% glycerol was added to the ejaculate, placed into sterile cryovials (0.75ml), held at-10 to-25C for 5 minutes, then placed into liquid nitrogen (LN) vapor. For washed sperm, the semen was diluted 1:1 with Ham's F-10 medium with 5mg/ml human serum albumin (HSA) and 0.01mg/ml gentamicin, followed by 10 minute centrifugation at 280-320xg. The pellet was resuspended in half the volume of Ham's F-10 (+ HSA and gentamicin) and an equal volume of the Ham's F-10 with 15% glycerol was added before adding to cryovials (0.5ml). Vials were placed into LN vapor for 30 minutes, followed by storage in LN. A test vial was thawed the following day. The data was expressed as the mean (± S.D).

Results: Of 165 specimens, 106 were unwashed and 59 washed, resulting in mean of 7 (4) unwashed and 3 (2) washed vials. Of 133 patients, 32 provided more than 1 ejaculate, 2 provided 6 specimens each. The parameters for the unwashed and washed group respectively were as follows: age 38.3 (6.4), 38.96 (5.6); volume 3.1 (1.7), 3.4 (1.7); count (million/ml) 63.6 (47.2), 72.97 (37); motility 57% (19), 62% (15); post-thaw count 34.3 (25.9), 65.7 (27.6); post-thaw motility 32% (15), 20% (10); motile concentration (million) per vial 9.6 (8.3), 6.9 (4.3).

Conclusions: Banking sperm before an IVF cycle is a reasonable option for men who might not be available for their partner's cycle, or who might have difficulty collecting at the scheduled time. In our practice, most specimens were prepared unwashed, presumably so the embryology lab could process the specimen. The preferable method for preparing frozen sperm for use in IVF appears to be without pre-freeze washing since post thaw motility and motile count per vial was higher in the unwashed group.

Disclosures: None.

Funding: None.

The Effect of Daily Ejaculation on Semen Parameters and Sperm DNA Damage in Normal Men

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Objective: Poor semen quality and sperm DNA damage have been associated with deleterious effects on male reproductive potential, including decreased fertility rates and increased rates of miscarriage. Although increased days of abstinence may have a negative effect on semen quality, the effects of increased frequency of ejaculation on semen quality have not been extensively studied. Here we assessed the effects of daily ejaculation on standard semen parameters and sperm DNA damage in normal men.

Design: Prospective study with subject as his own control.

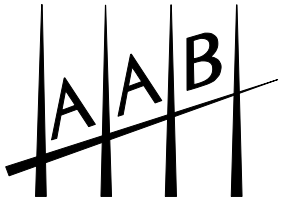
Materials and Methods: Twenty healthy men, without history of smoking or infertility, mean age 25, underwent daily ejaculations for 14 consecutive days after a 3-5 day abstinence period. Semen samples were collected on Days 1, 3, 7 and 14. Standard semen parameters were assessed, plus: 1) DNA integrity via fragmentation index (DFI) and high DNA stainability (HDS) using flow cytometry/acridine orange (SDFA) test, and 2) reactive oxygen species damage, oxidative stress adduct (OSA) test.

Results: Complete data on 20 men were obtained. Nineteen men began with normal semen parameters by WHO 2009 criteria. One subject demonstrated oligospermia. Significant decreases ($p<0.05$) compared to day 1 were observed in semen volume and total motile sperm count and a significant increase in mean DFI (14.1% vs. 15.3%; $p = 0.02$) and mean OSA (3.2% vs. 3.5%; $p = 0.03$) by day 14. No differences were observed in sperm c oncentration, motility, morphology, and immature sperm (HDS). Four subjects at baseline had abnormal DFI or OSA, three of whom normalized in either DFI or OSA by day14.

Conclusions: This study represents one of the most extensive examinations of semen parameters with daily ejaculation in normal men. Our study demonstrated that in normal men, daily ejaculation for up to 14 days produced expected reductions in semen volume and total motile sperm count. Significant increases in mean DFI and mean OSA were demonstrated by day 14, but not into abnormal ranges. The clinical significance of the increases in DFI and OSA is unclear. There was a subset of subjects with abnormal baseline DFI or OSA who actually improved into the normal range after 14 days of daily ejaculation. Some men with abnormal baseline sperm DNA integrity may benefit from daily ejaculation. Future studies will focus on men with compromised sperm DNA integrity at baseline who may improve with daily ejaculation.

Disclosures: Nothing to disclose.

Funding: None.



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