Abstracts Presented In 2009

Oral Presentations
Friday, June 5, 2009
3:00 p.m.-5:00 p.m.

Blastocyst Vitrification And Warming: Two Years And 200 Cycles

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Introduction: Vitrification has evolved to be a reliable procedure for preservation of oocytes and embryos during in vitro fertilization. Its efficiency, coupled with excellent survival rates has led to rapid assimilation of the technique into embryology laboratories. Numerous methods are available which have varying degrees of technical difficulty. This study evaluates the results of over 200 cycles of vitrification and warming using the cryotip.

Objective: The aim of this study was to evaluate embryo survival and implantation rates for patients having blastocyst vitrification within the first 2 years following the introduction of the procedure.

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

Results: The study evaluated 202 cycles of vitrification and warming resulting in the transfer of 391 embryos from March 2007 through December 2008. From 443 blastocysts vitrified, 94% (418) were recovered during warming and 94% (391) of these survived and were available for transfer. Pregnancy and implantation rates were similar for patients using donor or autologous oocytes (see table).

Discussion: This study shows that vitrified blastocysts survive and implant at high rates after warming and transfer. Although vitrification can be a technically challenging procedure, it is a reliable method for preserving blastocysts. The move toward elective single embryo transfer absolutely requires a solid cryopreservation program, and vitrification has emerged as a very reliable method for storage of surplus embryos.

	Own Oocytes	Donor Ooocytes	Total
Cycles	126	76	202
Embryos transferred (mean/patient)	250 (2.0)	141 (1.9)	391
Pregnancies (sac on ultrasound)	65	31	96
# sacs	79	42	121
Clinical pregnancy rate (%)	52	41	48
Implantation rate (%)	32	30	31
Twins (%)	8/65 (12)	8/31 (26)	16/96 (17)
Triplets (%)	2/65 (3)	1/31 (3)	3/96 (3)

Clinical Experience With Global® Medium As A Single Use, Complete Human Blastocyst Culture Medium

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Introduction: The reformulation of Global® medium (Biggers et al., 2004) produced a more stable complete medium with a longer shelf life and reduced ammonium production overtime. As a single-use embryo culture medium, Global® (LG) offers greater ease of use compared to sequential media systems in respect to incubator management, pH/Lot variations and culture/ET dish set-up/ patient. The objective of this study was to assess the overall effectiveness of LG medium as a dedicated blastocyst (BL) culture system.

Material and Methods: In 2008, 6956 human oocytes were surgically recovered from 524 ET patients by six different physicians. Cumulus oocyte complexes (COCs) were temporarily held in P1 medium (Irv. Sci., Santa Ana, CA) + 5% HSA until ICSI was performed. COCs were stripped in hyaluronidase solution (80 u/ml) and washed in LG-HEPES + 10% SS prior to ICSI. Post-ICSI, all oocytes were cultured in LG medium + 5% HSA in 25 µl droplets in 60x15mm Nunc culture dishes under oil (6.5ml, Global®). Tri-gas mini Sanyo (MCO-5) incubators were used, varying the $\%CO_2$ set point (5.0-6.3%) monthly to achieve a desired media pH based on stage of development and media Lot#. Normal 2PN zygotes and viable unfertilized eggs were isolated into a new culture dish on Day 1 and again on Day 3 following embryo

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morphological evaluation. Embryo transfers were performed on either Day 3 or Day 5 by transvaginal ultrasound guidance and the use of Sureview™ Wallace® catheters. One physician preferred Day 3 ETs, while the other physicians preferred Day 5 ETs, when justified.

Pt. Age	ET	#Pts	μ #/ET	+b-hCG (%)	Clinical Preg. (%)	Ong. Preg./LB (%)	# BL AA-BB (%)
Donor	D5	53	2.2	41 (77)	38 (72)	36 (68)*	469/711 (66)
≤ 34yo	D5	88	2.1	64 (73)*	57 (65)*	48 (55)*	602/1039 (58)
35-37yo	D5	49	2.5	34 (69)*	32 (65)*	29 (59)*	312/529 (59)
38-40yo	D5	40	3.1	28 (70)*	21 (53)*	17 (43)*	216/423 (51)
41-43yo	D5	12	4.0	9 (75)*	7 (58)*	3 (25)*	57/101 (56)
Donor	D3	16	2.7	13 (81)	12 (75)	9 (56)	N/C
≤ 34yo	D3	61	3.2	35 (57)	33 (54)	26 (43)	N/C
35-37yo	D3	63	3.4	32 (51)	29 (46)	22 (35)	N/C
38-40yo	D3	76	3.6	43 (57)	33 (43)	19 (25)	N/C
41-43yo	D3	66	3.3	16 (24)	14 (21)	6 (9)	N/C

*Different (P<0.05) than the corresponding D3 transfer group. N/C=not calculated.

Results: Pregnancy outcomes across age groups and Day of transfer (see Table.

High levels of good-excellent quality BLs were produced using donor eggs, with higher pregnancy success (i.e., live births) performing Day 5 BL ETs. Higher pregnancy rates were also observed across all age groups when performing D5 vs D3 ETs. Independent of age, we observed >50% viable BL production (transferable/ freezable quality) in non-donor egg patients. BLET occurred in 242 patients (46%) who produced a mean of 17.8 eggs/ pt. compared to 9.4 eggs/D3 pt. Pregnancy loss by Day of ET, within age groups, was similar (P>0.1), except in women >38yo due to significantly higher early pregnancy rates in the BLET groups (70-75% vs 57-24%). The latter effect was likely due to improved embryo selection. Excluding donors, 2PN fertilization rates also were elevated in the D5 ET group (78-81% compared to 65-71%) indicating potentially better egg quality.

Conclusion: Global® medium proved to be a reliable and high performance BL culture system. The trends and differences observed between D5 and D3 ET groups are similar to those reported in the literature. Single media culture system offers distinct advantages in QC and clinical application issues by minimizing factors that may adversely influence pregnancy success.

Complex Protein Supplementation Improves Control Rate Cryopreservation And Post-Thaw Re-Expansion Of Murine Blastocysts

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Introduction: While several studies have indicated supplementation of embryo culture medium with a globulin-rich protein source benefits embryo compaction and blastocyst formation, the affects of such complex proteins on cryopreservation and thaw solutions on blastocyst post-thaw survival is unknown.

Objectives: The purpose of this study was to test whether the supplementation of freeze and thaw solutions with the globulin-rich, commercial protein source Serum Substitute Supplement (SSS, Irvine Scientific) would improve post-thaw survival as measured by blastocyst re-expansion and total cell counts.

Materials And Methods: Frozen two-cell murine embryos were thawed and cultured in vitro to the blastocyst stage. Only cavitating blastocysts with discernable inner cell mass (ICM) and trophectoderm were considered for control rate cryopreservation. Freeze and thaw solutions (BlastFreeze and BlastThaw, Vitrolife) were supplemented with either SSS (10mg/ml HSA + 2mg/ml globulins; n=56) or human serum albumin (HSA, 10mg/ml; n=56). Following thaw, embryos were cultured for 24 hrs in G2v5 PLUS (Vitrolife) medium containing HSA (5mg/ml) or supplemented with SSS (5mg/ml). Blastocysts with >50% blastoceol re-expansion were considered as survived. Re-expanded blastocysts were fixed on slides, stained with DAPI, and viewed using epifluorescent microscopy to determine total cell counts. Data were analyzed using chi-square test and student's t-test.

Results: A greater number of blastocysts exhibited post-thaw re-expansion if frozen and thawed with solutions supplemented with SSS compared to blastocysts frozen and thawed in solutions containing only HSA (85.7% vs 66.1%; P<0.05). Total cell counts did not significantly differ between treatments, however, there was a trend supporting greater total cell numbers from re-expanded blastocysts that were frozen and thawed with solutions supplemented with SSS compared to blastocysts frozen and thawed in solutions containing only HSA (80.0 vs 70.5; P=0.057).

Conclusions: Our data demonstrate that the inclusion of a globulin-rich protein source, such as SSS, in freeze and thaw solutions improves blastocyst cryopreservation post-thaw survival.

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Embryo Development After Thaw Is Highly Predictive Of Pregnancy Outcome Regardless Of Stage Of Cryopreservation

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Objective: Cryopreservation provides an additional opportunity to achieve a pregnancy after an IVF/ICSI treatment. It is well accepted that continued embryonic development is an important criterion for embryo selection for ***fresh*** embryo transfer (ET). We believe that embryo development post-thaw may be equally predictive for pregnancy outcome in cryo/thaw cycles. Our purpose, therefore, was to assess the effect of continued embryo development, in addition to overall embryo quality, post-thaw and immediately prior to frozen ET (FET).

Materials and Methods: A total of 676 FET cycles, performed in our centre from January, 2006 to December, 2007, were retrospectively analysed for this study. The best quality embryos were selected for fresh ET, based on universally published criteria. Generally, remaining grade 1 and 2 embryos were frozen on days 3, 4 or 5. Day 3 and 4 embryos were placed in culture medium containing 1.5M DMSO and slowly frozen using a controlled step-wise method. For any subsequent cryo/thaw cycle, embryos frozen on day 3 or 4 were thawed quickly at room temperature, placed in a 0° C water bath for 10 min. and then transferred to medium containing consecutively decreasing levels of DMSO. These embryos were allowed to develop overnight before transfer. Day 5 embryos were frozen and thawed according to Menezo and were generally transferred later that same day. Pregnancy following FET was defined as a positive urinary test 14 days after ET. Progression of pregnancy was monitored using transvaginal ultrasound at 4, 7 and 10 weeks following transfer. Differences between groups were analysed and compared. Values with $p < .05$ were considered to be significant. Select data are expressed as mean ± SD.

Results: Part 1***:*** Initially, 2 groups of patients were analysed: Those who received 1 or 2 embryos demonstrating development prior to FET (**+DEV**; n=434) and those receiving 1-2 **good** quality embryos (primarily grade 1-2), but ***not*** displaying further development (**-DEV**; n=61). Embryo survival was greater than 50% in both groups. Mean number of embryos at ET were 1.24±.43 and 1.41±.5 for the **+DEV** and **-DEV** groups, respectively. Overall, pregnancy rate/ET was higher in the **+DEV** group (25.1 vs. 8.2% for **-DEV**; p<.006). Implantation (IR), IR +heart beat (+HB) and ongoing IR (ONG) were also higher in the **+DEV** compared to the **-DEV** group (*IR:* 19.9 vs. 6.6%; *+HB:* 18.1 vs. 2.6%; *ONG:* 17.0 vs.1.6%, respectively; p<.007). Delivery rates (deliveries/ET) were also higher (18.0 vs. 1.6%; p<.006). There were 12.4% twins in the **+DEV** group. There was only 1 delivery in the **-DEV** group and it was a singleton. Part 2***:*** Both the **+DEV** and **-DEV** groups were further analysed for number of embryos transferred (single: *SET* and double: *DET*). The **+DEV/** *DET* patients (n=179) had the highest PR compared to **+DEV/***SET* (n=255) (33.0 vs.19.6%, p< .003), but IR, +HB and ONG rates were not different. Delivery rates were also higher in **+DEV/**DET group (27.4 vs. 15.7%; p<.005). This group had a twinning rate/delivery of 22.4% vs. 0% for the **+DEV/**SET patients. In the **-DEV** groups (*DET* or *SET*), there were no ongoing pregnancies with *DET* and only 4.3% after the *SET* transfers (with only 1 delivery after 46 ETs).

Conclusions: Transfer of cryopreserved embryos that failed to develop after thaw resulted in such extremely poor ongoing pregnancy and delivery rates that the transfer of these embryos, at all, should be thoroughly discussed with patients. Alternately, transfer of embryos with continued development, regardless of embryonic stage at freezing, is highly predictive of a positive outcome.

Methamphetamine Exposure May Stimulate Fertility Potential As Assessed By The Hemi-Zona Assay

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Mammalian sperm carry receptors for many neuro-chemicals, including drugs of abuse such as nicotine, cocaine and methamphetamine (Meth). Sperm respond to cocaine because of the presence of membrane-bound opioid receptors; sperm function is altered by marijuana via cannabinoid receptors. Mammalian sperm also have receptors for neuro-chemicals that are affected by Meth: dopamine, epinephrine, norepinephrine and serotonin. The aim of this study was to investigate changes in sperm fertilizing potential using the Hemizona assay (HZA) and sperm that were treated in vitro with Meth.

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We hypothesized that in vitro Meth would produce significant changes in the ability of sperm to bind to the zona pellucida.

Semen samples were obtained from 5 donors from our screened donor pool. Samples were washed using Ham's F-10 (0.3% HSA), with and without 10 nM Meth. The pellet was layered with medium and a swim-up was performed. Aliquots of the supernatant were capacitated for 6-10 hours. Control portions were treated with Ham's F-10 only. Each supernatant was first analyzed using CASA (count, motility, velocity, head movement and hyperactivation). For the HZA, a matching pair of hemizonae were exposed to control or test sperm and the number of bound sperm determined after a 4 hour incubation. The number of sperm tightly attached was compared using the Hemizona Index. Chi-squared analysis was performed, using $p < 0.05$ as the cut-off.

Methamphetamine Stimulates Tight Binding of Sperm to Human Hemizona (HZ)

Control sperm (# bound/HZ)	Methamphetamine treated sperm (# bound/HZ)	Hemizona Index	Percent stimulation
71.6	171.6	244	144

The number of sperm bound to the outer zona was stimulated by 144% compared to the control hemizona ($p < 0.03$).

Meth users are commonly multi-drug users, have more frequent sexual encounters, more risky sexual behavior and a higher incidence of HIV infection. Short term exposure of sperm to Meth significantly stimulated fertilizing potential by increasing sperm hyperactivation and binding to the zona. If a male with HIV infection still has good health and if he is a light Meth user, he may have intense sexual cravings while on Meth and he may have enhanced fertility. Unwanted pregnancies are a possible result. These studies as well as future work will begin to create a better understanding of reproductive implications in methamphetamine addiction and new behavioral approaches for limiting the risks.

Ovarian Hyperstimulation Syndrome (Ohss) Does Not Compromise Embryonic Development And Pregnancy Outcome In A Subsequent Frozen Blastocyst Transfer Cycle

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Objective: As a management strategy for OHSS, elective cryopreservation of all produced embryos has been shown to be effective in the prevention of the late onset OHSS. However, in almost all the published reports, elective embryo cryopreservation for patients at high risk of severe OHSS were performed at pronuclear stage. Developmental potential of produced embryos is still unknown for patients at risk of severe OHSS. Effects of OHSS on blastocyst formation and pregnancy outcome of a subsequent frozen embryo transfer were investigated in this study.

Materials and Methods: Fresh embryo transfer was cancelled for 21 patients at high risk of severe OHSS from Jan 2004 through Dec 2008. Normally fertilized oocytes were cultured for up to 6 days in Sage Advanced Sequential culture system. Blastocysts graded as 3BB or better were frozen and thawed using Menezo 2-step slow freezing and thawing protocols. Patient returned to initiate a frozen embryo transfer at least 3 months after blastocyst cryopreservation. Fresh blastocyst transfer for patients without high risk of severe OHSS (n=350) during the same period was used as control. Blastocyst formation and pregnancy outcome were compared between fresh blastocyst transfer and frozen blastocyst transfer for patients at high risk of severe OHSS.

Results: A total of 84 frozen blastocysts from patients at high risk of severe OHSSwere thawed in 37 frozen transfer cycles, 70 survived (83.3%). Of the 72 frozen-thawed blastocysts transferred, 26 implanted (36.1%), resulting in a clinical pregnancy rate of 48.6% (18/37). Although patients at high risk of severe OHSS had higher numbers of oocytes retrieved and fertilized per cycle, no significant differences existed in patient age, rates of blastocyst formation, positive hCG,

Table 1. Embryonic development and pregnancy outcome between fresh blastocyst transfer of patients without risk of OHSS and the first frozen blastocyst transfer cycle for patients at high risk of severe OHSS.

Group	Fresh blastocyst transfer	The first frozen cycle for patients at risk of OHSS
Number of retrieval cycle	350	21
Mean age	34.1 ± 3.2	33.5 ± 3.7
Number of oocytes retrieved	17.0 ± 6.4	25.5 ± 5.9
Number of fertilized oocytes	12.0 ± 4.6	16.8 ± 5.9
Blastocyst formation	51.9% (2181/4203)	51.8% (183/353)
Embryos transferred per cycle	2.0 ± 1.5	1.9 ± 0.6
Rate of positive HCG	66% (231/350)	59.5% (22/37)
Clinical pregnancy rate	54.6% (191/350)	48.6% (18/37)

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and clinical pregnancy between fresh blastocyst transfer for patients without risk of OHSS and the first transfer cycle of patients at high risk of severe OHSS (all *P*>0.05) (Table 1.).

Conclusions: Our results show that OHSS does not comprise embryonic development and clinical outcome of a subsequent frozen embryos transfer. Due to the high developmental potential of a blastocyst, it is cost-effective to cryopreserve all embryos at blastocyst stage rather than pronuclear stage. Further study is needed to evaluate the cumulative pregnancy rate of elective blastocyst cryopreservation for patients at high risk of OHSS.

Relationship Between Seminal Plasma Heavy Metals And Sperm Function In Art

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Prior studies suggest that cadmium (Cd) may affect sperm fertility as a pro-oxidant leading to the generation of reactive oxygen species (ROS) and oxidative stress, while others report that infertile men have lower semen parameters and higher levels of ROS. The data below describes the competition between another pro-oxidant, inorganic lead (Pb) and zinc (Zn; an anti-oxidant) in three populations.

A prospective analysis of 140 male partners of couples undergoing their first IVF cycle demonstrated an inverse relationship between seminal plasma Pb and Zn. These metals have opposing effects on sperm count, IVF fertilization rate, mannose receptor expression and mannose-induced acrosome loss. Since IVF fertilization rates following dose compensated IVF insemination are unrelated to sperm count (n = 96, r = 0.139, NS), we focused on effects on a marker of human sperm function. Mannose receptor expression is a marker for IVF fertilization rates (r = 88, n = 0.449, P<0.0001; threshold predicting reduced fertilization [≤63%] = ≤30%), which is independent of sperm count (n = 95, r = 0.122, NS), motility (n =95, r = 0.132, NS) or normal morphology (n = 88, r = 0.082, NS). A seminal plasma Zn threshold was then determined using ROC curve analysis that best predicted poor IVF fertilization outcome (<0.515 mM).Using this threshold, we found that 2/3 of the outliers could be understood in light of the second metal. We found that in 7/12 with normal fertilization rates and elevated lead, seminal plasma Zn levels were above the Zn ROC curve threshold. In addition, we found that in 5/6 cases of reduced fertilization and normal lead levels, seminal plasma Zn levels were well below the zinc ROC curve threshold. To determine whether Zn and lead have similar effects in other populations, we examined the ranges of these metals in seminal plasma from donors participating in an artificial insemination program (AI) and in unselected men from the general population. The data show that the ranges of Zn and Pb were similar between IVF patients, normal fertile semen donors, and unselected male population at large, while cadmium levels were significantly elevated in IVF patients ($p < 0.0001$). Seminal plasma Zn and Pb were inversely correlated in AI donors (n = 12; r = -0.786; $p < 0.025$) and also have opposing effects on AI pregnancy rates using donor sperm and on pregnancy by coitus. The IVF threshold for seminal plasma Zn (≤ 0.515 mM) was positively correlated with pregnancy by AI (n = 12; r = 0.648,; p< 0.03) and by coitus (n = 9; r = 0.862; $p < 0.0001$). Mannose receptor expression was also correlated with pregnancy following donor AI (n = 12; r = 0.765; $p < 0.015$) and with pregnancy by coitus (n = 13, r = 0.781, $p < 0.005$).

These results suggest that Zn supplementation may offer an inexpensive oral therapy for the negative effects of Pb on male reproductive function.

TLR3-Induced Genes In An In Vitro Implantation Model

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Successful implantation involves reciprocal interaction between the embryonic trophoblast cells and the epithelial lining of the endometrium. Unraveling the signal transduction involved in such interaction may lead to new strategies to correct implantation failure and improve pregnancy rates, especially in the IVF setting. In ungulates, type I interferon signaling is critical for successful implantation, but the role of IFN signaling in primates and rodent implantation remains unclear. We previously observed an increase in Toll-like receptor 3 (TLR3) expression in human endometrium during the implantation window and demonstrated that ligation of endometrial TLR3 results in strong induction of type I interferon production. Therefore, we hypothesized that TLR3 action may

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be important in the early dialogue between embryo and endometrium.

Investigation of early embryo-endometrial interaction *in vivo* is limited by both ethical and methodological barriers. Therefore, we developed an *in vitro* model allowing manipulation and study of embryo-endometrial interaction. The endometrial epithelium was modeled using a monolayer culture of human uterine epithelial cell line, RL95-2, grown on a thin layer of growth-factor depleted matrigel After 20 hours of RL95-2 cells culture on matrigel, a monolayer was evident and mouse blastocysts (live or dead) were carefully placed on the cells without disrupting the monolayer. Alternatively, the RL95-2 monolayer was treated with a TLR3 ligand, Poly I:C or a negative control PolydI:dC. The RL95-2 cells were harvested and total RNA was extracted after 8 hours. The effects of embryos or TLR3 ligand on expression of inflammatory genes thought to be involved in embryo implantation were measured using Taqman real time (RT-PCR). Measurement of constitutively-expressed genes, GAPDH and PPIA were used to normalize relative expression data.

We observed that Interferon β (IFN-β), Leukemia Inhibitory Factor (LIF), Indolamine 2,3-Dioxygenase (IDO, enzyme that degrades tryptophan) and RANTES (CCL5, chemoattractant for monocytes, T helper cells and eosinophils) were strongly induced by Poly I:C stimulation, but only mildly induced by the live embryos or dead embryos at 8 hours. Further studies, including a dose response of the embryos and a time course are underway to confirm these findings; however the data suggest that signaling events in humans during implantation may be similar to events triggered by interferons in other species.

Poster Presentations
Friday, June 5, 2009
6:15 p.m.-8:15 p.m.

Buffering Capacity And Osmolarity Of Culture Media Used In Assisted Reproductive Procedures Are Not Affected By Expiration

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Objective: Fertility laboratories across the globe heavily rely on the utilization of commercially available culture media for in vitro fertilization (IVF) procedures. The manufacturers specify short expiration duration, usually 3-4 weeks, thus indirectly forcing end users to discard a considerable amount of unused media. The IVF procedure cost can be controlled if media waste can be prevented. Nutrients as well as the environmental stability are the two criteria that affect media quality. In this study we investigated if the two important components of the medical environment such as its buffering capacity and osmolarity are compromised by expiration.

Design: The pH and osmolarity of expired culture media were compared with that of unexpired and unused ones in different conditions.

Materials and Methods: Three sequential culture media, Q-fert, Q-cleve and Q-blast manufactured by SAGE Assisted Reproductive Products Inc. (Trumbull, Connecticut) were investigated under unexpired and expired conditions for pH and osmolarity changes. In the unexpired group, the measurement was performed in original condition (first time opened). The expired group underwent more than one opening along with the removal of variable amount of media for procedural use, and stored for different duration of time before they were used in the study. Manufacturers suggested instruction was followed in operating the pH meter (Orion 410+, Thermo Electronic Corporation) as well as the osmometer (vapor pressure osmometer model 5100C, Wescor Inc., Logan, UT). The osmolarity was measured at ambient condition. The pH was evaluated at ambient, and at 37º C in 5.5% CO_2 maintained incubator environment under overnight incubation. A total of 12 unexpired and 85 expired media bottles were analyzed. Statistical analyses were performed where appropriate.

Results: The expired Q-fert, Q-cleve and Q-blast media at ambient exhibited significantly ($p < 0.005$) higher pH compared to that of corresponding unexpired-unused controls (7.77 ± 0.15 vs 7.29 ± 0.03, 7.75 ± 0.15 vs 7.20 ± 0.01, and 7.72 ± 0.12 vs 7.27 ± 0.03). The expired media showed a significant negative correlation (Q-fert: $r = -0.51$, $p = 0.005$; Q-cleve: $r = -0.63$, $p = 0.001$ and Q-blast: $r = -0.50$, $p = 0.003$) pattern reflecting lower volume and higher pH. On the contrary, a significant positive correlation (Q-fert: $r = 0.77$, $p = < 0.001$; Q-cleve: $r = 0.63$, $p = 0.001$ and Q-blast: $r = 0.63$, $p = < 0.001$) implicating longer expiration duration and higher pH was seen in expired media. Both the unexpired and expired media groups

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exhibited similar pH values (Q-fert 7.37 vs 7.40, Q-cleve 7.29 vs 7.33, and Q-blast 7.32 vs. 7.35) under 24 hours CO_2 incubation indicating retention of full buffering capacity. Under incubation, neither media volume ($r = 0.19$, $p > 0.5$) nor expiration duration ($r = 0.23$, $p > 0.5$) hold any correlation with the media pH. The osmolarity readings of expired media fluctuated but were within the manufacturer's provided accepted range (257 – 273 mOsm).

Conclusions: These results suggest that 1) although the pH of media get elevated by expiration but such elevated pH can be stabilized back to normal by CO_2 incubation, and 2) no significant impact of expiration on osmolarity as it fails to push osmolarity out of the physiological range. Our finding encourages investigation of nutritional status of expired media to see if such media can be considered for use at least in case of an emergency need.

Elective Single Embryo Transfer Is A Viable Option For First Cycle Patients <35 Years Old Receiving Day 3 Transfers

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Objective: To determine if Day 3 (D3) Elective Single Embryo Transfer is a viable option for patients <35 years old undergoing their first cycle of IVF, who do not meet criteria for Day 5 (D5) transfer.

Design: A retrospective study comparing pregnancy outcomes of 87 autologous, first cycles of patients <35 years of age; 24 patients chose Elective Single Embryo Transfer (eSET) and the remaining 63 patients chose to transfer 2 embryos (eDET). All patients underwent their first cycle of IVF between January 1, 2007 and March 24, 2009, and did not meet criteria for D5 transfer. All pregnancies are either delivered or are still ongoing.

Materials and Methods: Embryos were cultured in Sage media in 6% O_2. All patients included had <6 Top quality embryos on D3 (1/1/2007-6/8/2008) or <4 Top quality embryos on D3 (6/8/2008-3/24/2009), deeming them ineligible for D5 transfer. A Top quality embryo is defined as one with the following characteristics at the indicated time points post-insemination: 25 hours: Between the 2 pronuclear stage and 2 cell stage; 42-44 hours: 4-5 cells, even; 66-68 hours: ≥6 cell,even with <20% fragmentation. Pregnancy is defined as one which is delivered or still ongoing. Pregnancy rates were compared using Chi-square.

Results/Conclusions:

- No significant difference in pregnancy rate was seen between eSET and eDET on Day 3.
- eDET lead to a significantly higher rate of twin pregnancies.
- Transfer of 2 Top embryos led to a significantly higher twin pregnancy rate ($) without increasing overall pregnancy rate (#).
- Transfer of 2 Non-top embryos produced a higher pregnancy rate than transfer of 1 Non-top embryo, but the difference did not reach significance (^). There was no increase in twin pregnancies.

Discussion: This data supports recommending the following for patients <35 years old with Day 3 transfers on their first cycle of IVF when they do not meet criteria for Day 5 Transfer:

- ❖ Transfer of only 1 Top embryo.
- ❖ Transfer of 2 Non-top embryos.

Comparison of Pregnancy Rates for Elective Single Embryo and Double Embryo Transfer

	Pregnancy Rate (Delivered or Still Ongoing)	% Twin Pregnancies
eSET	45.8% (11/24) *	0% (0/11)[&]
eDET	52.4% (33/63) *	48.5% (16/33)[&]
	*P=0.59	[&]P= 0.004

Influence of Embryo Quality on Elective Single Embryo and Double Embryo Transfer

	Total # Embryos Transferred	# Top Embryos Transferred	Pregnancy Rate (Delivered or Still Ongoing)	% Twin Pregnancies
eSET	1	0	25.0% (1/4)[^@]	0.0% (0/1)
	1	1	50.0% (10/20)[#@]	0.0% (0/10)[$]
eDET	2	0	42.9% (3/7)^	0.0% (0/3)
	2	1	42.9% (9/21)	44.4% (4/9)[+]
	2	2	60.0% (21/35)[#]	57.1% (12/21)[$+]

[#]P=0.47; ^P=0.55; [@]P=0.37

[$]P=0.002;[+]P=0.52

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Microsecure Vitrification (µS-Vtf) Of Mouse Blastocysts: Comparison To S³ Vitrification Using 0.25 Ml Straws Or Cryopettes®

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Introduction: Previously, we validated a novel VTF technique in the mouse model (Schiewe and Fahy, 2008), that we now refer to as a microSecure (µS) device. In combination with the S³ VTF system (Stachecki et al., 2008), the µS-VTF system offers sterility and security using FDA compliant devices, in addition to being safe, simple and successful in clinical application. The aim of this study was to confirm the effectiveness of the µS-VTF system compared to the S³-VTF system, and to test the technical efficacy of the new Cryopette® (CP) device.

Materials and Methods: Using frozen-thawed 1-cell mouse embryos (Embryo Tech, MN), 100 expanded to hatching blastocysts (BLs) were vitrified in each experiment using either the µS or S³-VTF system (Expt. 1) or contrasting the µS to the CP device in Expt. 2**.** All methods used S³ VTF solutions (V1-3: 5 min, 5min and 1 min dilutions, respectively; and T1-5: see below). The µS-VTF technique loaded BLs into shortened (cut: 2.0 cm) denuding pipettes (275-300 µm ID), detached, sterile wiped, loaded tip-end first and then LN_2 plunged /stored in sealed CBS™ High Security straws. Upon thawing, internally sealed ID rods are safely read while the vitrified contents remained submerged in LN_2. Once confirmed, the inner plug end was rapidly cut upon removing the straw and the VTF tip is simply poured into 15 ml of 1.0M sucrose solution (37°C; 60x15mm culture dish). Within 10-15 sec, the VTF tip contents were expelled into the T1 solution and serially pipette with the VTF tip through T5 at 5 min intervals (T1-4 at RT, T5 at 37°C) before placement in a Global® microdroplet culture system. The S³ BLs were pipette directly into labeled 0.25 ml straws, sealed and placed in a LN_2 vapor tank at -100°C for 2 min before storage in LN_2. For thawing, S³ straws were held at room temperature for 5 sec and submerged in a 37°C water bath for 10 sec before expelling contents into T1 solution. S³-treated BLs were then diluted and cultured as described above. The Cryopette® (CP; Mid-Atlantic Diagnostics, NJ) is a finely drawn plastic micropipette (5 cm long) connected to a bulb device, which upon sealing the tip creates a closed system. The bulb was completely depressed to load V3 solutions and the embryos before easily heat-sealing the tip prior to direct LN_2 plunge. BL survival was assessed and continued BL development recorded at +24 hr.

Results: In 10 replicates of 5 BLs each/treatment, no difference was observed in recovery rates (100%) or survival rates (100%) in Expt. 1 or 2. Continued BL development in Expt. 1 decreased 10-18% overnight, but was not different (P>0.05) between the µS- and S³-VTF systems (90% and 82%, respectively).

Conclusion: The µS-VTF system proved to be highly effective, similar to the established S³ VTF system. Both VTF systems offer technical simplicity aimed at reducing intra- and inter-laboratory variation, as well other quality control advantages compared to various VTF devices. The S³ system is a macro-VTF approach using 0.25 ml straws, which are standard to the IVF industry. The microSecure VTF device incorporates commonly used denuding pipettes with optimum cryosecurity. Both systems have been successfully applied in clinical IVF to definitively disprove the theory that ultrarapid cooling rates are necessary for the successful VTF of human eggs and blastocysts. The novel CP device proved to be reliable and easily applied by four different embryologists with high survival rates. The CP device requires additional viability studies and further product development to overcome QC concerns in labeling and cryostorage that ensures ease of identification and the safety of the tip, respectively.

Support: We thank T. Fortino of Mid-Atlantic Diag., Inc. for allowing us to test the CP device.

Next Generation Quality Control In The Ivf Laboratory: Using Data Loggers To Monitor Real Time Temperatures Of Laboratory Equipment

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Quality Control (QC) in the IVF laboratory plays an important role in the success of any IVF program. The role of QC procedures in the IVF laboratory is to fine tune existing protocols in order to more effectively help infertile patients in their quest to have a healthy baby. The three most

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important physical conditions in the IVF laboratory that can be controlled are temperature, pH and osmolality. Laboratories are required to document and monitor these physical conditions regularly. Monitoring of temperatures inside incubators, refrigerators and freezers is an integral part of routine day to day QC in the IVF laboratory and is a prerequisite for accreditation by CAP, JCAHO or other agencies. In laboratories with multiple banks of incubators and freezers and liquid nitrogen dewars this is a time consuming and laborious process. In large volume laboratories this process can take up to 1 hour every day. Here we report an improved and simpler way to meet this QC requirement by using data loggers which continuously transmit temperature of incubator, freezer and refrigerator interiors at set intervals of 4.16 minutes or less, directly on to a PC. Maximum and minimum values over a period of 24 hours, high and low temperature alarm notification via phone or email were programmed into the system, documented directly and saved as files. We studied one such data logger from Marathon Products, San Leandro CA, the RF2 data logger with its accompanying MDAS-PRO software and adapted this to the IVF laboratory. The RF2 system is NIST certified and transmits temperature data as a graph and as real time data points. Drifts and spikes, temperature changes when doors are opened and recovery times were monitored and documented continuously in an efficient manner. Incubators were recalibrated in cases of drift, using multiple data points each reading 4.16 minutes apart, for a tighter control of acceptable ranges. Recovery times for temperatures inside the incubators to stabilize each time the doors were opened ranged between 43 to 64 minutes. During the normal course of an IVF procedure on day 0 of the procedure, the incubator doors were being opened around 6-7 times and temperatures fluctuated between 1.2- 0.4 C below set temperature of 37 C. Inter technician variation was bypassed and productivity in the laboratory was increased by saving 260 hours of labor in terms of technician time per year which resulted in a saving between $6500 to $7800 annually. Data logging enabled us to pinpoint the exact time and durations of changes in incubator, freezer or refrigerator interior temperatures and is by far a more efficient system to monitor these fluctuations than single data point measurements currently in use. Continuous recording of interior temperatures over a period of months allowed for closer monitoring of the environment in which embryos were being cultured and prompt trouble shooting when drifts from the norm occurred. Accuracy and precision of the RF2 probes compared well with the ASTM E-77 and NIST certified mercury thermometers. In conclusion data logging provides easy, hands off approach to quality control in the IVF laboratory and is a time saving procedure that is reliable and convenient.

Prognosis For Clinical Pregnancy And Delivery After Transferring Embryos Obtained From A Cohort Of Incompletely Mature Oocytes At Retrieval Time

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The objective of our retrospective study is to establish prognosis for an implantation, pregnancy and delivery rate following transfer of embryos originating either from 1) immature oocytes (GV and MI stage) with overnight in vitro maturation or 2) from mature oocytes and overnight in vitro matured oocytes that were transferred together.

Total of 159 IVF cycles (1999-2008) were extracted from our database where ICSI was performed on mature oocytes at retrieval time and/or on oocytes that matured overnight. There were 24 cycles (out of 159) where embryo transfer was not performed because there were no embryos available for transfer due to lack of fertilization or cleavage. The remaining 135 cycles were grouped as follow; Group LM (n=50 late mature oocytes) - all transferred embryos originated from the oocytes that matured after overnight culture of GV and MI stage oocytes. Group MX (n=43, mixed) – embryos from both the mature oocytes and inseminated on day of egg retrieval as well as embryos from the oocytes that matured after culturing in vitro overnight and inseminated on the second day. Group M (n=42, mature) all transferred embryos originated from retrieved mature oocytes and inseminated on the day of egg retrieval.

There were statistical differences between these three groups (LM, MX and M) 1) in the average number of oocytes retrieved (6.30, 8.63, 11.62, $p<0.02$,), in average % of mature oocytes on day of egg retrieval (32.9%, 57.5%, 69.1%, $p< 0.001$), in average number of 2 PNs (0.07, 1.79, 4.24, $p<0.0001$), in the number of cleaved embryos originated from mature oocytes on day of egg retrieval (0.036, 1.63, 3.90,

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p<0.001), respectively. There were no statistical differences between the three groups (LM, MX, M) in the number of oocytes that matured after overnight culture and had been inseminated on second day or in the number of 2PNs from these oocytes. However, the cleavage rate of the embryos originated from the oocytes cultured overnight in the mature group (M, 46.8%) was significantly lower (p<0.0001) then in the other two groups LM (94.2%) and MX (97.6%). Clinical pregnancy, delivery and implantation rates for group LM was 11.36%, 11.36%, 5.6%, for group MX was 13.95%, 11.63%, 4.17% and for group M was 45.24%, 33.3% and 14.61% respectively.

Conclusion. Overnight culture of immature oocytes (GV and MI stage) resulting in next day maturity and transferable embryos might be clinically beneficial in situations where no mature oocytes are retrieved. In spite of low implantation, pregnancy and delivery rates, some patients nevertheless may have a chance for a positive outcome. Therefore, in this selected population of patients with GV and MI stage oocyte maturity at time of retrieval, overnight maturation may result in competent embryos for transfer.

Training Graduate Students In The Genomic Age: The Unique Challenges Of Molecular Diagnostic Pathology

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Since the inception of the Texas Tech University Health Sciences Center (TTUHSC) graduate program in Molecular Pathology in 2003, the development of the curriculum has been a fluid and dynamic process to meet the demands of this developing profession. The curriculum is assessed annually by the following methods: advisory committee, input from faculty retreat, feed back from clinical preceptorship sites, surveys from employers, survey from graduates, and outcome measures. The evaluation process over the past six years has lead to the development of a curriculum that has moved away from a research emphasis to a practice management emphasis. The clinical research component has been redefined to comprehensively address start up testing and assay validation. In addition, a course has been developed that specifically concentrates on the unique challenges of the operational issues involved with accreditation, personnel development, and external communication with clinicians. A human genetics course was added in place of an introductory molecular diagnostics course, which has deepened the level of graduate study. The redevelopment of a statistics course which now includes relevant human genetic statistics and finally, adding a cell biology course, which has acted as a leveler to the diverse student enrollment. Currently, we are working to integrate the courses to provide the students a more congruent experience. The design of the curriculum for the 21st Century molecular pathology degree program must include components that prepare graduates for the demands of start up testing and assay validation as well as unique management issues related to the diagnostic molecular laboratory.