embryos in each group (73% vs 76%, p=0.40). Both the average number of frozen embryos (5.0 ± 5.6 vs 5.0 ± 5.0, p=0.99) and the average number of frozen embryo cycles per patient was similar between ever-users and never-users (1.7 vs 1.5, p=0.133). No significant difference was seen in per frozen cycle live birth rates (35.0% vs 39.0%, p=0.40) or cumulative live birth rates (50.9% vs 51.4%, p=1.0). Finally, no difference was seen in rate of frozen transfers resulting in spontaneous abortion (14.5% vs 10.7%, p=0.33).

CONCLUSION: Smoking has been linked to delayed conception, increased rates of infertility and earlier menopause. We were unable to demonstrate a significant decline in outcomes in either fresh or frozen cycles in our population of ever-users. Any significant detriment to IVF outcomes from tobacco usage may be a result of duration related or dose dependent toxicities. Changes in personal history data collection at our institution over the study period limited data analysis in this regard. Another limitation is the inherent potential bias of self-report personal history questionnaires, although the proportion of ever-smokers in our population approached that commonly stated for the general female population in the USA.

Supported by: None.

P-84

Pre-Freeze Manual Collapsing of the Blastocele Cavity Increases Cryosurvival of Human Blastocysts. M. C. Schiewe, F. Miller, G. D. Hubert, R. P. Buyalos. Fertility and Surgical Assoc. of CA, Thousand Oaks, CA.

OBJECTIVE: Improvements in the cryosurvival of human blastocysts (BL) have been made principally by strict embryo selection (i.e., good to excellent BL quality) and by using enhanced commercial cryodiluents. Recently, we reported the effective use of CBS(Cryo Bio Systems, France) straws for cryopreserving human BL, with a 31% live birth rate. We have noticed that hatching BL and post-PGD hatched BL are prone to pre-freeze collapse, but have high rates of post-thaw survival. The purpose of this study was to prospectively collapse BL, using a manual technique, prior to conventional freezing in an attempt to decrease water volume in the blastocele cavity, thus reducing potential cryodamage to the trophectoderm.

DESIGN: Apriori Design, Prospective Analysis.

MATERIALS AND METHODS: Embryos were produced in a sequential media combination of Enhanced D3/IVC-2, Advantage Cleavage Medium and G2.3/CCM with supplementation of 10% SS and 125ug/ml HA, as necessary, using microdroplets under oil. Embryos were cultured in a humidified, CO2 gas (5.0%-6.0%) atmosphere maintained at 37°C. In this study, pre-freeze BL handling involved either standard methodology (control) or a blastocele collapsing treatment. BL were collapsed during cryodilution by repeated serial pipetting/agitation, beginning with a Stripper P-200, then a P-150 and finally a P-135, if necessary. BL stage embryos were diluted twice in commercial BL freezing solutions (glycerol based, F1 to F2 at 10 minute intervals; Irvine Scientific, Santa Ana, CA) before being loaded into CBS straws (1 or 2 BL/straw), seeded at -7°C, slow cooled to -35°C and stored in liquid nitrogen. High quality (QG=AA) BL were selected for cryopreservation, with lower quality (QG=AB, BA or BB) sibling BL included per patient freeze cycle. Upon thawing, straws were held horizontally in room air for 30 sec, wiped dry and placed into a 37-38°C water bath for 30 sec before expelling and recovering the BL(s). Cellular contraction and or lysis of the BL was assessed after multi-step dilutions of glycerol (1.0-0.1M) and sucrose (0.4-0.05M) in H-HTF+10%SS to estimate viability. BL were cultured for 3-18 hrs, examined for blastocele reexpansion and transferred.

RESULTS: Between Sept. 2004 and March 2005, 40 patients had BLs thawed. 31 patients had received conventional pre-freeze embryo handling/cryodilution (n=95 BL), while 9 others employed a BL collapsing treatment (n=27 BL). Manual collapsing of the BL prior to freezing increased ($P<0.05$) initial cryosurvival (n=25 survived, 93%) and BL re-expansion (n=22, 80%) before ET, compared to control BL (n=66 survived, 69%; n=58 re-expansion, 61%). Comparing patients under 38 years old, no difference in pregnancy rates was detected for control (10 of 25, 40%) or treated BL (2 of 5, 40%).

CONCLUSION: Manually collapsing BL before cryopreservation is not detrimental to their subsequent post-thaw viability. In fact, less embryo wastage occurs by employing pre-freeze blastocoel collapsing, with greater BL re-expansion. Although manually collapsed BL are arguably more viable post-thaw, our preliminary data does not reveal a difference in pregnancy outcome.

Supported by: None

P-85

Blastocyst Cryopreservation: To Slow-Freeze or to Vitrify? J. Liebermann, E. Knopoff, J. Matthews, A. Erman, S. Sanchez, M. Tucker. Fertility Centers of Illinois, Chicago, IL; Georgia Reproductive Specialists, Atlanta, GA.

OBJECTIVE: Since the first report of a birth of a baby from a frozen embryo transfer (FET) approximately 250,000 babies from FET have been born. Clearly, cryopreservation techniques have become a widely used routine procedure as an increasingly important therapeutic strategy in assisted reproduction. As an alternative to the widely used slow cooling method of human blastocysts there has been some recent publications using an ice-crystal-free cooling method for biological material called vitrification. Objections to cryopreservation aside, vitrification is very simple, potentially faster, inexpensive, fairly well established and more consistent than conventional cryopreservation. Furthermore, vitrification has another advantage in that it is time flexible and therefore allows blastocysts to be cryopreserved at their optimal stage of development and expansion without the necessary time required for slow cooling methods.

DESIGN: A retrospective analysis comparing two different cryopreservation techniques.

MATERIALS AND METHODS: One year's experience with vitrification (VIT) of blastocysts utilizing the Cryotop system of vitrification (Kitazato, Japan), with a mixture of 15% ethylene glycol/DMSO (v/v) + 0.5M sucrose, yielded the following results in comparison with contemporaneous data using the conventional slow cryopreservation (CONV) with 10% glycerol + 0.2M sucrose. All closed Cryotops were secondarily stored inside 5ml liquid nitrogen pre-filled cryovials.

RESULTS: It is clear to date that blastocyst cryosurvival (SURVIVAL) is improved following vitrification: 224/232 (96.6%) vs. 358/395 (90.6%) (p=.075, NS). With increasing numbers of cryopreservation cycles, this is translated in to improved overall clinical outcomes: Clinical pregnancy rate (CLIN PR) 50.0% vs. 47.4% (p=.15, NS); Viable pregnancy rate (VIABLE PR) 46.2% vs. 42.3% (p=.10, NS); and Implantation rates (IR) based on heartbeat was 30.6% vs. 24.9% (p=.055, marginally significant).

Cryo Method	Age	# Thaw Cycles	# Embryos thawed	# Embryos survived	Mean # Embryos @ Transfer	Clinical PR/Thaw (%)	Viable PR/Thaw (%)	IR (%)
Vit	34.2	106	232	224	2.1	50.0	46.2	30.6
Conv	35.0	175	395	358	2.0	47.4	42.3	24.9

CONCLUSION: Even without statistical significant clinical improvement, it is quite evident that after vitrification the cryosurvival is more consistent, allowing greater ease of patient management with transfers (ET) being almost certain to occur. With much shorter procedural protocols, vitrification can be undertaken on a more flexible basis by laboratory staff, and reduces personnel time commitment. Vitrification is now our standard protocol for cryopreservation of human blastocysts within our program.

Supported by: None

P-86

Suboptimal Embryo Morphology on Day 3 Does Not Predict Poor Pregnancy Potential. K. M. Silverberg, H. Werland, T. Turner, C. Schuster, R. Fields, T. Vaughn. Texas Fertility Center, Austin, TX; Austin IVF, Austin, TX.

OBJECTIVE: Most IVF programs transfer the morphologically "best" (Tier 1) embryos on Day 3 of a fresh cycle, while other good quality (Tier 2) embryos may be frozen at that time. Embryos that are neither morphologically normal enough to be transferred fresh nor frozen on Day 3 (Tier 3) continue in culture - frozen on Day 5 or 6 only if they become blastocysts.

This study was designed to assess the pregnancy potential of Tier 3 embryos.

DESIGN: Prospective analysis of all patients undergoing FET in 2004 in a large private infertility practice.

MATERIALS AND METHODS: Oocytes were inseminated in Sage fertilization medium with 0.5% HSA and cultured to Day 3 in Sage cleavage media with 10% SPS. Tier 1 embryos were transferred fresh on Day 3; remaining 6-cell or greater grade 1-2 (Tier 2) embryos were frozen on Day 3; all other (Tier 3) embryos were transferred into Sage blastocyst media with 10% SPS for continuing culture. Tier 3 embryos were reevaluated on Days 5 and 6 and frozen only if they became good quality blastocysts. Statistical analysis was performed using t-test and chi square analysis.

RESULTS: 116 patients underwent FET in 2004. Embryos included in this study were frozen between 2002-2004, and no significant protocol changes occurred during that interval. Five patients had no embryos survive thaw. Of the remaining 111 patients, 61 (55%) achieved pregnancy, with 36 (32.4%) ongoing or delivered at this time. Seventy-three patients underwent transfer of 192 Tier 2 multi-cell embryos, while 38 patients received 76 Tier 3 blastocysts. Although cell survival percentage was greater in Tier 3 embryos, ongoing/delivered pregnancy rates were not different (32.8% Tier 2, 31.6% Tier 3). While the implantation rate (IR) per embryo thawed was greater in Tier 3 embryos, there was no difference in IR/embryo transferred.

	Tier 2	Tier 3	P value
# Embryos Thawed	3.7	2.1	<0.05
# Embryos Transferred	2.6	2.0	NS
Cell Survival (%)	48.3	73.4	<0.05
Ongoing/Delivered Preg/ET (%)	32.8	31.6	NS
Implantation/Embryo Thawed (%)	11.5	15.1	<0.05
Implantation/Embryo Transferred (%)	16.7	17.1	NS

CONCLUSION: Tier 3 cryopreserved embryos afford the same chance for pregnancy as do Tier 2 cryopreserved embryos. As embryo morphology on Day 3 predicts neither suitability for ultimate cryopreservation nor pregnancy potential, ART programs should culture Tier 3 embryos to Day 5-6 and crypreserve those embryos that achieve the blastocyst stage. Patients should be counseled that these "leftover" (Tier 3) embryos still afford a legitimate opportunity for a successful pregnancy.

Supported by: None

P-87

High Survival and Hatching Rates of Bovine Blastocysts Following Vitrification. J. Y. Huang, S. L. Tan, R. C. Chian. McGill University, Montreal, PQ, Canada.

OBJECTIVE: Cryopreservation of embryos by slow freezing is not entirely consistent. Reliable methods of cryopreserving different stages of embryos for most species are still under development. The objective of this study is to evaluate the survival rate (SR) and hatching rate (HR) of bovine blastocysts following vitrification in order to introduce this methodology for human ART.

DESIGN: Animal model study.

MATERIALS AND METHODS: Bovine blastocysts were produced from abattoir materials following IVM-IVF-IVC. On day 7 of embryo culture, total of 190 blastocysts were divided into two groups: 1) Control (n=96): the embryos were cultured continually until day 9; 2) Vitrification (n=94): the blastocysts were cryopreserved by vitrification method and stored at -196°C at least for 1 week, and then the frozen blastocysts were thawed and cultured for 2 days. SR and HR were compared between these two groups. As the procedure of vitrification, the blastocysts were suspended in equilibration solution (7.5% ethylene glycol and 1,2-propanediol) for 5 min, and then the blastocysts were transferred to vitrification solution (15% ethylene glycol and 1,2-propanediol + 0.5 M sucrose) for 45-60 seconds at room temperature. The blastocysts were loaded on a special designed vitrification tool, Cryoleaf, and immediately plunged it into liquid nitrogen (LN2) for storage. For thawing, the Cryoleaf was directly inserted into 37°C thawing solution (1.0 M sucrose) for 1 minute. The thawed blastocysts were transferred to 0.5 M and 0.25 M sucrose solutions for 3 minutes respectively, and then washed twice with blastocyst culture medium for culture. To determine the numbers of non-viable cells in the blastocysts following vitrification, a total of 62 blastocysts (Control: n=35 versus Vitrification: n=27) were examined by the terminal deoxynucleotidyl transferase mediated dUTP nick-end labeling (TUNEL) assay. TUNEL assay was performed on day 8 (post-thawing 1 day of culture) according to the users' manual. The blastocysts were examined under an epifluorence microscope using a FITC filter and a DAPI filter. Non-viable cells of each blastocyst were counted out of the total number of cells. Percentage data were subjected to arcsin transformation and the transformed data were analyzed by ANOVA using the SAS statistical software package (SAS, 1990). Comparison of means among groups was performed using Duncan's Multiple Range Test.

RESULTS: SR of bovine blastocysts was 100% (94/94) following vitrification. There were no differences in HR of bovine blastocysts between control (62.5% = 60/96) and vitrification (61.7% = 58/94) groups. The number of non-viable cells in the blastocysts was not significantly different ($P>0.05$) between control (5.0 ± 2.9) and vitrification (9.5 ± 4.0) groups.

CONCLUSION: Blastocysts can be successfully vitrified and this method may be efficiently used for cryopreservation of human embryos

Supported by: None

P-88

Postthaw Embryo Compaction is Predictive of the Success of Frozen-Thawed Transfer. H. Wang, A. Le, R. Boostanfar, M. Feinman, B. Behr. Huntington Reproductive Center, Westlake Village, CA.

OBJECTIVE: Although cryopreservation of supernumerary embryos has increased the overall success rate in human IVF-ET, the survival and implantation rate of frozen-thawed embryos is directly related to the quality of embryos at the time of freezing. Overnight incubation improves selection of good quality embryos for transfer. Studies showed that there was a ~45% implantation rate for day 4 embryos after fresh morula ET. This study is designed to investigate the predictors of postthaw embryo compaction and the relationship to the outcome of frozen-thawed embryo transfer (FET) cycles.

DESIGN: Retrospective analysis of frozen embryo transfer (FET) cycles conducted during 2001-2003 at a clinical IVF center.

MATERIALS AND METHODS: A total of 186 human pronuclear embryos from 35 patients and 754 day 3 embryos from 137 patients were cryopreserved using a Freeze Control (CL-863) freezing unit (Biogenics, Napa Ca) and the standard Testart/Lasalle 1.5M PrOH/0.1M Suc freeze/thaw procedure, with the modification of starting the freeze at-6.5°C. On thawing, embryos were cultured *in vitro* in Cleavage (for pronulcear embryos) or Blastocyst medium (for cleavage embryos) (Sage) with 10% Serum Substitute Supplement (Irvine Scientific). Embryo transfer was performed at 48~54 hours and 24~30 hours, respectively, for the pronuclear and cleavage stage from time of thaw into a recipient uterus after standard estradiol/progesterone preparation. Significant differences were assigned at $p<0.05$.

RESULTS: The survival rates of the pronuclear and cleavage embryo were 91% and 86.5%, respectively in all age groups. Embryo transfer was performed in 160 patients and produced a clinic pregnancy rate of 53.1% in various age women. The clinic pregnancy rate of patients who had compacted embryos at the time of transfer was 77.4% (24/31), while the clinical pregnancy rate was only 47.3% (61/129) for patients who had no compacted embryos for transfer ($P<0.01$). For the embryo survival rate, and the number of embryo transfer, there were no significant differences between these two groups ($P>.05$).

CONCLUSION: Extending the culture of thawed pronuclear and cleavage stage embryos allows for better embryo selection, resulting in higher pregnancy and implantation rates per FET. Postthaw embryo compaction is strong positive predictor for the success of frozen-thawed transfer.

Supported by: None

P-89

The Efficacy of Assisted Hatching on Pregnancy Rates of Cryopreserved-Thawed Embryo Transfer at the Cleavage Stage. L. Sun, S. Chen. Nan-Fang Medical University, Guangzhou, Guangdong, China.