DESIGN: Retrospective analysis comparing Fresh-DO transfer cycles to those where the oocytes were cryopreserved and thawed prior to fertilization and transfer (ET).

MATERIALS AND METHODS: Oocyte donors were stimulated and retrieved in routine fashion. Oocytes were either used fresh or cryopreserved (half by slow-cooling, half by vitrification) 2h post-retrieval. Surviving frozen-thawed as well as fresh oocytes were fertilized using ICSI and the resultant embryos were cultured to the blastocyst stage; based on morphologic assessment, embryos were selected for ET. Prior to ET, fresh recipients underwent a GnRH-agonist synchronized estrogen/progesterone replacement cycle while frozen recipients underwent a programmed estrogen/progesterone replacement cycle. Following ET, standard luteal monitoring was performed.

RESULTS: Between 2006 and 2008, 88 anonymous Fresh-DO cycles and 12 Frozen-DO thaw cycles were performed. Cycle outcomes are listed.

	Fresh-DO Cycles	Frozen-DO Thaw Cycles	P
Donor Age	26.0 ± 3.2 y	28.4 ± 4.2 y	.02
# Oocytes Assigned to the Recipient	1226 (13.9 ± 7.2 eggs)	126 (10.5 ± 2.7)	.004
# MII	827 (9.7 ± 4.8)	116 (9.7 ± 2.0)	1.0
# 2pn	782 (8.8 ± 4.3)	75 (6.3 ± 1.7)	.001
# of embryos transferred	2.0 ± .45	2.2 ± .39	.1
IR	45% (78/172)	46% (12/26)	.7
CPR	57% (50/88)	75% (9/12)	.2
LBR	53% (47/88)	64% (7/11) *	.4

Data is mean ± SD *1 pregnancy was ongoing at the time of submission.

CONCLUSION: Despite a lower number of available 2pn zygotes in Frozen-DO thaw cycles, cycle outcome data was not compromised. In fact, pregnancy and live birth rates (although not significant) were greater than in Fresh-DO ICSI. This data supports more widespread use of frozen donor oocytes, a practice that would reduce donor cycle costs and screening limitations, and allow for more equitable distribution of oocytes, ultimately changing the practice of female gamete donation.

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VIABILITY OF VITRIFIED-THAWED DAY 3 HUMAN EMBRYOS FOLLOWING 24 HOUR CULTURE AND TRANSFER. E. S. Michael, M. T. Lao, A. Michael, T. Michael, T. Tao. Astra Fertility Clinic, Mississauga, ON, Canada.

OBJECTIVE: Embryo selection is arguably the most important factor influencing frozen-thawed embryo transfer cycle outcomes. The purpose of this study was to examine whether culturing embryos for 24 hours before transfer allows for better discrimination between viable and non-viable embryos.

DESIGN: Retrospective study.

MATERIALS AND METHODS: Vitrification and thawing were performed using Medicult medium system and standard operating procedures. Whenever possible, embryos were thawed until an average of 3 (range 1-6) survived (with ≥ 50% of the original blastomeres remaining intact). Transfer was performed approximately 24 h after embryo thawing. According to the number of embryos that grew (increased their cell numbers) after 24 h culture, patients were divided into four groups: A, with none or only one embryo that grew; B, with two embryos that grew; C, with three embryos that grew; and D, with four to six embryos that grew. Statistical analysis was performed by using the chi-square test.

RESULTS: Results are shown in Table 1.

Outcome of embryo thaw procedure between groups with different numbers of embryos that grew after 24 hour culture

Patient Groups	A	B	C	D
Embryos survival rate	56% (37/66)[a]	90% (132/146)[b]	94% (266/284)[b]	92% (130/142)[b]
Embryo growth rate	41% (15/37)[a]	74% (98/132)[b]	94% (249/266)[c]	92% (119/130)[c]
Pregnancy rate	10% (2/21)[a]	26% (13/49)[a]	44% (37/83)[b]	43% (12/28)[b]

[a,b,c] Values with different superscript letters within the same row are significantly different (P<0.05).

CONCLUSION: The results of our study suggest that 24 hour in-vitro culture of vitrified-thawed day 3 embryos may be beneficial to assess their true developmental potential, even though the embryos survive well after thawing. High embryo growth rate (with at least 3 grown embryos per frozen embryo transfer cycle) after 24 hour culture seems to be necessary to achieve pregnancy. This may have practical implications for patients who have a large number of frozen embryos, raising selection problems.

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EVALUATION OF ASEPTIC BLASTOCYST VITRIFICATION IN PREIMPLANTATION GENETIC DIAGNOSIS PATIENTS. L. Van Landuyt, E. Van den Abbeel, D. Stoop, W. Verpoest, P. Devroey, G. Verheyen. Centre for Reproductive Medicine, UZ Brussel, Jette, Brussel, Belgium.

OBJECTIVE: To analyse the outcome of closed blastocyst vitrification in preimplantation genetic diagnosis (PGD) patients.

DESIGN: One-hundred blastocyst warming cycles of PGD patients performed between April 2008 and February 2010 were evaluated retrospectively. Morphological survival was assessed according to blastocyst characteristics. Clinical pregnancy rate was evaluated in 78 single (SET) and 13 double embryo transfer (DET) cycles.

MATERIALS AND METHODS: Vitrification of supernumerary blastocysts biopsied on day 3 was performed using the closed CBS-VIT High Security straws in combination with the Irvine Scientific^{R} Freeze Kit. Early blastocysts were frozen on day 5. Full and expanded artificially hatching blastocysts were frozen on day 5 and day 6. Outcome parameters were compared using Chi-square test with $P < 0.05$.

RESULTS: The mean female age was 30.7 years. In total, 131 blastocysts were warmed with a post-thaw survival of 83.2% (109/131) and a transfer rate of 79.4% (104/131). Day 5 blastocysts survived better (90.4%, 75/83) than day 6 blastocysts (70.8%, 34/48, $p < 0.01$). No difference in survival rate was observed between early cavitating (89.2%, 33/38) and full/expanded blastocysts (93.3%, 42/45). Thirty-nine of the 45 full/expanded blastocysts were artificially hatching at the moment of cryopreservation. In 9 cycles, no blastocyst was available for transfer (1.0 warmed/cycle). The clinical pregnancy rate was 16.7% (13/78) after SET and 30.8% (4/13) after DET. Overall, the implantation rate was 16.3% (17/104) per embryo transferred. In SET, the implantation rate was 12.7% (7/55) for blastocysts frozen on day 5 and similar to the implantation rate of day 6 blastocysts (13.5%, 5/37).

CONCLUSION: Blastocysts derived from biopsied embryos can be successfully vitrified using closed vitrification. Day 6 blastocysts have a lower survival rate compared to day 5 blastocysts. However, when being transferred, similar implantation rates can be obtained.

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DONOR OOCYTE CRYOPRESERVATION: A RANDOMIZED CLINICAL TRIAL COMPARING MICROSECURE VITRIFICATION (μS VTF) TO CHOLINE-ENRICHED CJ3 SLOW-FREEZING (SF). M. C. Schiewe, N. Nugent, S. Zozula, J. J. Stachecki, R. E. Anderson. Southern California Institute for Reproductive Sciences, Newport Beach, CA; Southern California Center for Reproductive Medicine, Newport Beach, CA; Thyo-Galileo Research Laboratories, LLC, Livingston, NJ.

OBJECTIVE: The aim of this study was to randomly compare a SF and a novel VTF procedure within donor oocyte source in regards to survival, fertilization rates, embryo development and pregnancy outcomes.

DESIGN: Randomized a priori arrangement of cryo-treatments and batch assignment to prospective Oocyte Adoption couples for embryo transfer.

MATERIALS AND METHODS: This IRB approved study involved the harvesting of oocytes from 15 consenting unproven Egg Donors (21-27 y.o.) exposed to a standard COH protocol. The length of stimulation (10-16d) and hCG dosage (5000-10000iu) varied based on individual response. Oocytes were recovered, stripped and cryopreserved (3-5 oocytes/container) within 37.5-38 hr post-hCG. The μS VTF-treated oocytes were diluted in S3-Oocyte solutions in 3 steps (5min, 2min, 1min) and maintained in shortened (cut: 2.0 cm) sterile flexipettes (300 μm ID, Cook), detached, sterile wiped, and sealed into CBS™ High Security straws. SF oocytes were treated in 2-steps of CJ3 solutions, loaded

into CBS™ straws and cooled slowly (-0.3°C/min from -7°C to -35°C then plunged). All oocytes were thawed at room temperature in a series of sucrose dilutions. ICSI was performed on surviving oocytes +2hr post-thaw and cultured in LG medium+5%SS (tri-gas, 37°C). Chi squared analysis was performed.

RESULTS: 314 Donor oocytes were recovered, 259 cryopreserved (82.5% MII), and 217 thawed, to date. After 25 oocyte thaw cycles, 100% recovery was achieved, with survival and fertilization rates being higher ($P<0.05$) by μS VTF (87%, 82%) versus SF (45%, 59%). Pregnancy, ongoing/live birth and implantation rates also were higher by μS VTF (64%, 43%, 45%, respectively) than SF (29%, 14%, 13%, respectively).

CONCLUSION: μS VTF in S3-Oocyte solutions proved to be a more reliable technique than SF in CJ3 solutions for oocyte cryopreservation. Some variation in oocyte survival and embryo development seen in the μS VTF treatment is likely attributed to differences in cytoplasmic maturation/egg quality between donor sources.

Supported by: EMD Serono Grant.

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HIGH RECOVERY AND SURVIVAL RATES OF MOUSE AND HUMAN BLASTOCYSTS VITRIFIED IN A CLOSED SYSTEM. W. Wang, K. Kaskar, L. Gong, G. Haddad, I. Gill. Houston Fertility Institute/Tomball Hospital, Tomball, TX.

OBJECTIVE: To assess the recovery and survival rates of mouse and human blastocysts using a closed vitrification system.

DESIGN: Laboratory and clinical study.

MATERIALS AND METHODS: Mouse and human blastocysts were vitrified in a pre-cooled protective straw without direct contact to liquid nitrogen. In Group 1, mouse blastocysts were vitrified and warmed. In Group 2, abnormal human blastocysts from preimplantation genetic diagnosis (PGD) were vitrified and warmed. In Group 3, patient blastocysts were vitrified, warmed and transferred using the closed system. In Group 4, as a control, patient blastocysts were vitrified using Cryotop vitrification system. Recovery, survival and pregnancy rates were assessed.

RESULTS: In Groups 1 and 2, 80 mouse blastocysts and 45 abnormal human blastocysts all showed recovery and survival. In Group 3, transfer of 8 blastocysts from 4 patients resulted in 3 pregnancies (75%). In Group 4, transfer of 101 blastocysts from 62 patients yielded a pregnancy rate of 79%.

TABLE 1. Blastocyst survival and pregnancy outcome using closed system and Cryotop

Embryo sources	No. of cases	Vitrification system	No. of embryos warmed	No. of embryos recovered	No. of embryos survived	No. of blastocysts transferred	No. of clinical pregnancy
Mouse	4	Closed	40	40 (100%)	40 (100%)	NA	NA
Human(PGD)	14	Closed	45	45 (100%)	45 (100%)	NA	NA
Human	4	Closed	8	8 (100%)	8 (100%)	8	3 (75%)
Human	62	Cryotop	101	101 (100%)	98 (97%)	98	49 (79%)

CONCLUSION: The closed vitrification system developed in this study is a safe method for cryopreservation of human blastocysts without direct contact to liquid nitrogen during freezing and storage. This method provides full embryo recovery and high survival rates as compared with Cryotop method. Most importantly, it will reduce possible contamination between samples in cryostorage.

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THE PREGNANCY RATE IS SIGNIFICANTLY INCREASED WHEN THE DURATION OF PROGESTERONE SUPPLEMENTATION AND EMBRYONIC AGE ARE SYNCHRONIZED IN DAY 3 FROZEN-THAWED EMBRYO TRANSFER. E.-M. Cha, S.-K. Kim, J.-H. Lee, I.-H. Park, G.-H. Jeon, K.-H. Lee. Infertility Lab, MamaPapa & Baby OBGY, Ulsan, Republic of Korea; Department of OBGY, College of Medicine, Inje University, Haeundae Paik Hospital, Busan, Republic of Korea.

OBJECTIVE: To evaluate the difference of pregnancy rate according to the duration of progesterone(P) supplementation on day 3 Frozen-Thawed embryo transfer(FET).

DESIGN: Retrospective study.

MATERIALS AND METHODS: We analyzed 165 cycles from patients underwent FET from Jan 2009 to March 2010. All patients received high dose of estradiol valerate (6mg) to prevent premature LH surge, starting from day 1 or 2 of the menstrual cycle without pituitary down regulation. when the endometrium was developed sufficiently ($\geq$8mm) detected by ultrasound examination, patients were given 50-100mg of IM P in oil daily until serum β-hCG test was performed. Embryos were frozen and thawed on day 3 using a vitrification protocol. We divided our data into two groups based on the duration of P supplementation. In group 1 (n=35) progesterone was administered 3 days prior to FET day. In group 2 (n=130) progesterone was administered 4 days prior to FET day. FETs were performed on the same day of embryo thawed. Pregnancy identified through detection of G-sac in transvaginal ultrasound examination.

RESULTS: There was no difference between both groups in patient age (33.70 vs. 34.27), the mean number of transferred embryo (2.31 vs. 2.61), the mean number of cryopreserved embryo (8.19 vs. 7.63), and embryo quality. Both groups are in acceptable range of implantation window. However, the pregnancy rate was significantly higher in group 1 (37.1%) than group 2 (23.8%).

CONCLUSION: Progesterone induces morphological and biochemical changes in the endometrium for "implantation window" and after. But the precise duration of P supplementation for FET is still a matter of debate. Our study shows that pregnancy potential is higher when day 3 embryo is transferred on the same day of P supplementation. It seems that extended duration of P supplenmentation than embryonic age doesn't induce a better outcome in pregnancy rate. Thus we emphasize that synchrony between endometrial and embryonic development is a crucial factor for not missing implantation window.

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BEAD VITRIFICATION OF SPERMATOZOA FOR MEN WITH SEVERELY COMPROMISED SPERMATOGENESIS. J. C. Y. Hu, Q. V. Neri, Z. Rosenwaks, G. D. Palermo. The Ronald O Perelman & Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College, New York, NY.

OBJECTIVE: Successful vitrification of human spermatozoa without cryoprotectant (CP) has been reported but has been done in an open system with consequent exposure to liquid nitrogen (LN_2).

DESIGN: In this study, we attempted to vitrify sperm in small beads with and without CP in a closed system.

MATERIALS AND METHODS: Specimens ($n = 8$) with normal parameters were donated from consenting men. Each sample was equally distributed among three categories: semen suspension mixed with v/v dilution of CP (LCP), higher proportion of CP (HCP), or without CP (WCP). Each sample was either cryopreserved in vapor at -80°C (30min) before plunging into LN_2 or vitrified by dropping 10μl of specimen on a LN_2 pre-cooled glass slide. Sperm beads were transferred to a pre-cooled vial and plunged into LN_2. Control fractions were thawed by exposing vials them to room temp. Warming of individual beads was done by placing a bead on a pre-warmed (37°C) Makler.

RESULTS: From Dec 2003-Nov 2009, 872 semen specimens with an average density of $67.7 \pm 33 \times 10^6$/ml, motility $56.7 \pm 7\%$, and $4.2 \pm 2\%$ morphology were cryopreserved in standard fashion. Post-thaw motility of 30.5 $\pm$ 13% was obtained representing a 26.2% decrease. The study samples had a mean density of $66.4 \pm 34 \times 10^6$/ml, motility $51.8 \pm 6\%$, and $7.1 \pm 3\%$ morphology. Post-thaw of LCP was 35.4 $\pm$ 12%, while HCP was 38.4 $\pm$ 13%. When samples were vitrified, LCP post-motility was 31.3 $\pm$ 6% and HCP 27.8 $\pm$ 12%. Warming of WCP sperm beads yielded 33.0 $\pm$ 11% motility, LCP 31.3 $\pm$ 6%, and HCP 27.8 $\pm$ 12%. The decrease in progressive motility ranged from 13.4 to 24%.

CONCLUSION: Cryopreservation of semen in borderline asthenospermic men was unrelated to the CP concentration. Vitrification of raw semen surprisingly yielded comparable retention of motility. Vitrification in a closed system remarkably shortened cryopreservation time and the single bead assessment allowed expedited sampling of individual specimen sparing the remainder.

Supported by: Institutional, QVN is funded by CTSC-WCMC NIH grant ULI RR024996.