We evaluated two systems for the ease of the procedure, type of storage device, recovery rate post-warming and the cryo-survival rates.

DESIGN: Prospective analysis.

MATERIALS AND METHODS: 103 oocytes were donated by IVF patients who had given consent for research use of discarded tissues. Oocytes were retrieved 36 hours post-hCG, and were treated with hyaluronidase ~4 hours later for maturity assessment. Immature oocytes that matured to Metaphase II within 24 hours of retrieval were studied. 52 oocytes were vitrified/warmed using the Medicult Vitrification Cooling and Warming kits (Medicult, Denmark) with McGill Cryoleaves that exposed oocytes directly to Liquid Nitrogen (LN_2), and 51 oocytes utilized the Irvine Scientific Vitrification Freeze/Warm kits (Irvine Scientific, Santa Ana, CA) with CryoTips preventing direct LN_2contact. Oocytes were stored in LN_2 until warming. The number of oocyte recovered and surviving 3 hours post warming was assessed. Chi-square analysis was performed.

RESULTS: See table.

	Medicult (%)	Irvine Scientific (%)
MII vitrified/warmed	52	51
Oocytes unable to be recovered	2 (4)*	17 (33)*
Survival 3 hours post-warming	48/50 (96)**	27/34 (79)**

*, ** $p<0.05$.

CONCLUSIONS: Oocyte vitrification has emerged as a reliable cryopreservation method for human oocytes, as preliminary data show high cryo-survival. In our experience, oocyte recovery was significantly better using the McGill Cryoleaf ($p=0.0003$). We noted that a large number of CryoTips experienced fracture and breakage. The Medicult system produced a significantly higher oocyte survival rate ($p=0.04$). Overall the Medicult kits were easier, with fewer steps and the more user friendly McGill Cryoleaf, producing significantly better results. Evaluation of other vitrification kits with multiple alternate storage devices are underway.

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DOES HAVING SURPLUS EMBRYOS TO FREEZE SERVE AS A PROGNOSTIC INDICATOR OF IVF OUTCOME? M. Luna, G. Vela, B. Sandler, F. Arredondo, M. Duke, A. B. Copperman. Reproductive Medicine Associates of New York, New York, NY; Department of OBGYN and Reproductive Science, Mount Sinai School of Medicine, New York, NY; Reproductive Medcine Associates of Texas, San Antonio, TX.

OBJECTIVE: To evaluate whether having supernumerary embryos available for cryopreservation may be a marker for discriminating patients who produce embryos with greater implantation potential and thus have a better prognosis in their fresh IVF cycle.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: Patients <35 yrs with normal FSH (<10IU/L) who underwent a fresh ET from 12/02-03/09 were included. Cycles were classified by ET day (D3/D5), and subclassified based on blastocyst availability for cryopreservation. Independent of ET day, surplus embryos are assessed and cryopreserved on D5/D6 if morphologically optimal. Analysis of outcome including implantation (IR), clinical pregnancy (CPR) and loss rates was performed for each group.

RESULTS: 2071 ETs were identified, of which 990 were performed on D3 and 1081 on D5. Although the mean embryos transferred was similar between the cryo and no cryo groups for each type of transfer, significantly higher CPR and IR were noted for the subgroups having surplus embryos frozen.

	Day 3 ET n=990		Day 5 ET n=1081	
	CRYO n=216	NO CRYO n=774	CRYO n=674	NO CRYO n=407
Age	31.2±2.8	31.4±2.9	31.1±2.9	31.2±3.0
FSH (IU/L)	6.4±2.0	6.8±1.8	6.3±1.9	6.5±1.6
Number of ET	2.6±0.6	2.5±0.8	2.2±0.4	2.4±0.7
CPR	149/216 (69%)*	450/774 (58.1%)*	486/674(72.1%)~	225/407(55.3%)~
IR	247/561(44%)∧	702/1971(35.6%)∧	758/1447(52.4%)◇	334/987(33.8%)◇
Loss Rate	23/149 (15.4%)	49/450 (10.9%)	54/486 (11.1%)	21/225 (9.3%)

* $p=0.0051$, ~ $p=0.0336$, ∧ $p<0.0001$, ◇ $p<0.0001$.

CONCLUSIONS: Patients who have surplus high quality embryos frozen demonstrate higher CPRs in the fresh cycle from which these originated. We hypothesize that embryo cohort-specific variables exist, and that the reproductive competence of sibling embryos may predict developmental competence as measured by IVF cycle outcomes. Surplus blastocysts available for cryopreservation may be used as a prognostic indicator of outcome in young patients who have undergone an ET in the same cycle.

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SHIPPING CRYOPRESERVED EMBRYOS TO LONG TERM STORAGE: ARE OUTCOMES AFFECTED? S. Senn, F. Nehchiri, R. Artac, M. Dangcil, F. Rabara, D. Dasig. Kaiser Permanente Center for Reproductive Health - Fremont, Fremont, CA; Kaiser Permanente Center for Reproductive Health - Sacramento, Sacramento, CA.

OBJECTIVE: Relocating cryopreserved embryos to a long term storage facility is an attractive option for the IVF laboratory. Due to the benefits, including minimizing the liability for abandoned embryos and reducing the need for numerous storage tanks, both Kaiser IVF centers in CA chose to transport embryos to offsite storage. An assessment of this policy was completed.

DESIGN: Retrospective comparative study.

MATERIALS AND METHODS: The records for FET cycles between Oct.'08 and Mar.'09 were reviewed for survival rate (SR), implantation rate (IR) and clinical pregnancy rate (CPR). Embryos were frozen at the blastocyst stage or the 2PN zygote stage using standard slow freezing techniques in vials or straws. The outcomes of (Group A) FET cycles using embryos that were shipped to a long term storage facility (Reprotech Ltd, Reno, NV) and then shipped back to our facility were compared to the outcomes of (Group B) FET cycles that used embryos that were never shipped. All blastocysts frozen were at least of grade 3BB or better on day 5 or 6. All zygotes were frozen with visible pronuclei. Embryos were transported in dry LN2 shipping vessels by either Reprotech staff or FedEx. A cryo guard vial was used to indicate whether a rise in temperature of the tank occurred during shipment.

RESULTS: 13 FET cycles represented Group A. 37 embryos were thawed with a 85.2% SR, IR of 41.9% (13/31) and CPR of 61.5% (8/13). Group B comprised of 62 FET cycles resulted in a 78.2% SR, IR of 25.3 % (37/146) and CPR of 41.3 % (26/63). Clinical pregnancy is defined as the presence of a fetal sac by ultrasound at 6 weeks.

CONCLUSIONS: Transferring frozen inventory to a long term storage facility is a successful strategy to minimize burdens associated with in house storage. Our results demonstrate that out sourcing frozen embryo storage does not adversely affect the ability to achieve normal survival, implantation and clinical pregnancy rates. Future studies to include vitrified specimens are needed as vitrification methods become standard practice.

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CLINICAL EVALUATION OF THE CRYOLOCK® DEVICE AS A CARRIER FOR VITRIFICATION OF BLASTOCYST STAGE EMBRYOS OBTAINED FROM CRYOPRESERVED DONATED OOCYTES. D. P. Bernal, C.-C. C. Chang, T. A. Elliot, C. W. Elsner, A. A. Toledo, Z. P. Nagy. Reproductive Biology Associates, Atlanta, GA.

OBJECTIVE: To evaluate the efficiency of a new vitrification carrier (Cryolock®) by analyzing clinical outcomes, after warming and embryo transfer (ET) of vitrified blastocysts, derived from previously vitrified/warmed oocyte cycles from the donor egg bank.

DESIGN: Observational study.

MATERIALS AND METHODS: A total of 15 recipients (who were not pregnant after the initial oocyte warming treatment cycle) have had frozen embryo replacement on day 20 of a supplemented cycle (embryos originating from vitrified/warmed oocytes were cryopreserved at the blastocyst stage using Cryolock® (Biodiseño Ltda, Bogota-Colombia) as a carrier device); Blastocyst warming was performed by serial dilutions in three steps using 1.0M, 0.5M, and 0M sucrose solutions. Embryo transfer was performed 2-3 h after warming. HCG level was tested 10 days after ET, clinical pregnancy was confirmed by detecting fetal cardiac activity on the 7th week of pregnancy.

RESULTS: Blastocyst survival rate after warming was 95% (38/40), 36 embryos were transferred (average 2.4 per transfer), Twelve clinical pregnancies were obtained (80%), 19 embryos implanted (53%). Two first trimester

miscarriages have occurred, and seven pregnancies are currently ongoing and 3 deliveries were obtained by date (with 5 healthy babies - 3 females and 2 males, no abnormalities detected).

CONCLUSIONS: These results show that the Cryolock® device is a suitable carrier for embryo (re-) vitrification. Additionally, the pregnancy outcomes demonstrate that oocyte vitrification and embryo re-vitrification is an efficient technical and treatment approach, not impacting oocyte/embryo viability in a significant manner. High survival and implantation rates of the (double) cryopreserved embryos suggest that viability of oocyte and blastocyst is retained when using the combination of Cryolock® and vitrification technique.

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VALIDATION OF A NEW METABOLIC MARKER TO ASSESS THE VASCULAR VIABILITY OF VITRIFIED WHOLE EWE OVARIES. A. Torre, M. Momier, C. Mazoyer, D. Vertu-Ciolino, B. Salle, J. Lornage. U846 Stem-cell and Brain Research Institute: Reproduction, Epigenesis and Development, INSERM, Lyon, France; Medecine de la Reproduction, Hôpital Femme-Mère-Enfant, Hospices Civils de Lyon, Bron, France; Laboratoire de Biologie de la Reproduction, Université Claude Bernard Lyon-1, Lyon, France.

OBJECTIVE: To explore the pedicle metabolism of VS4 (cryoprotector avoiding ice formation in isolated ovarian pedicles but not in cortex) vitrified whole ovaries, by developing a drip of Thiazodyl Blue Tetrazolium (MTT) as metabolic marker.

DESIGN: *In-vitro* study.

MATERIALS AND METHODS: Pairs of ewe ovaries were perfused with MTT (1g/l) for 2 hours at 39°C. Group A: tissue lesions were induced by immersing ovaries into 53° or 65°C heated mediums or into liquid nitrogen, prior to MTT drip. Group B: different metabolic substrates (D-glucose, L-glucose) and inhibitor (2-deoxy D-glucose) were added to the MTT drip. Group C: ovaries were exposed to VS4 ± vitrified-thawed prior to MTT perfusion. Pedicle's MTT staining was either qualitatively analyzed on frozen sections (histological assessment) or solubilized in alcohol and quantified by optical density at 564 nm. Statistics: ANOVA, $p<0.05$ considered significant. Each experiment compared the MTT staining of 10 treated ovaries with their paired controls.

RESULTS: MTT stained strongly and reproductively vessel's smooth muscle. Liquid nitrogen, 53°C or 65°C tissue lesions significantly reduce MTT staining by 93.9%, 48.0% and 94.3%, respectively. MTT could thus be considered as a viability marker. MTT staining partially depended on D-glucose metabolism: its inhibition by 2-deoxy D-glucose, privation of D-glucose or its replacement by unmetabolised L-glucose significantly reduce MTT staining by 23.6%, 40.9% and 31.6% respectively. While thawing, ice seeding from ovarian cortex, constantly invaded the fully VS4 vitrified pedicles (recrystallisation): they significantly stained 29.9% less than control, with histological focal unstained areas, whereas unvitrified VS4 exposed pedicles stained like controls.

CONCLUSIONS: Thawing is critical for incompletely vitrified whole ovary. Thus MTT appears to be a proper marker to evaluate whole organ cryoprotection protocols.

Supported by: Agence de Biomédecine, Fondation de l'AVENIR.

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THE USE OF SIBLING OOCYTES TO COMPARE OOCYTE VITRIFICATION OUTCOMES WITH DIFFERENT CRYOPROTECTANT COMPONENTS: A FORMULA WITHOUT DMSO. C.-C. Chang, D. P. Bernal, T. A. Elliott, S. M. Slayden, D. Mitchell-Leef, Z. P. Nagy. Reproductive Biology Associates, Atlanta, GA.

OBJECTIVE: Dimethyl sulfoxide (DMSO) has been widely used as a cryoprotectant for oocyte cryopreservation. However, it has been demonstrated that DMSO could potentially trigger oocyte activation. Thus, our objective was to compare oocyte vitrification outcomes with vitrification solution containing DMSO vs. without DMSO.

DESIGN: Prospective study.

MATERIALS AND METHODS: Twenty-eight recipients with an average age of 41.5±5.5 yr were matched to use donor eggs from the egg bank (16 donors: average age of 26.9±2.5 yr). Cryopreservation on donor sibling oocytes was performed by two methods: cryoprotectants containing 15% ethylene glycol, 15% DMSO and 0.5 M sucrose (group A) and cryoprotectants containing 30% ethylene glycol and 0.5 M sucrose (group B). Sibling oocytes from both groups were warmed for each recipient. Oocytes were fertilized by ICSI 2-3 hours after warming. On day 5, blastocyst formation was assessed and embryo transfer was performed. Results were analyzed by the Chi-square ($P<0.05$) test.

RESULTS: A total of 196 MII oocytes were warmed for 28 recipients (7.0±2.35). One hundred twenty-six oocytes were warmed from group A (4.5±1.75), and 70 oocytes were warmed from group B (2.5±0.83). Survival rates were 90% (113/126) and 94% (66/70; NS) in A and B, respectively. Fertilization rates were 82% (103/126) in A, and 83% (58/70; NS) in B. Blastocyst formation rates were 65% (82/126) and 57% (40/70; NS) in A and B. Embryos for transfer were selected from group A (35) and B (21); 56 blastocysts were transferred to 28 recipients (2.0±0.38); 18 out of 28 had positive hCG (64%). Thirteen recipients had a clinical pregnancy (22 FCAs detected; 39% implantation rate), and all 13 pregnancies are ongoing.

CONCLUSIONS: We demonstrated that the survival, fertilization, and blastocyst rates were similar when use sibling oocytes to compare vitrification solution with and without DMSO. It suggests a cryoprotectant solution without DMSO can provide equivalent efficiency of oocyte vitrification to a common protocol that contains DMSO.

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FROZEN BETTER THAN FRESH? A MATCHED-COHORT STUDY COMPARING AUTOLOGOUS CYCLES USING FRESH AND THAWED EMBRYOS. B. S. Shapiro, S. T. Daneshmand, F. C. Garner, M. Aguirre, C. Hudson, L. R. Kern. Fertility Center of Las Vegas, Las Vegas, NV; Obstetrics and Gynecology, University of Nevada School of Medicine, Las Vegas, NV.

OBJECTIVE: Compare fresh blastocyst cycles to cycles using blastocysts derived from post-thaw extended culture (PTEC) of thawed bipronuclear (2pn) oocytes.

DESIGN: Matched cohort study.

MATERIALS AND METHODS: Following controlled ovarian stimulation with gonadotropins, oocytes were inseminated by ICSI. Indications for cohort cryopreservation included premature luteinization or history of implantation failure. Thawed 2pn oocytes were cultured to the blastocyst stage before transfer. PTEC cycles were matched against fresh cycles by matching on age at retrieval and total number of 2pn oocytes. Patients were <41 years old at retrieval and each had 2pn oocytes derived from one retrieval.

RESULTS: The 172 PTEC cycles were matched to 172 fresh cycles. The two groups were similar in age, number of 2pn oocytes, and day 3 FSH level. The cancellation rate was greater in the PTEC group than the Fresh group (22.8% vs 8.7%, respectively, P=0.0009). However, the implantation rate (54.4% vs 35.1%, P<0.0001), ongoing pregnancy rate per transfer (65.6% vs 43.9%, P=0.0002) and ongoing pregnancy rate per retrieval (51.2% vs 40.1%, P=0.049), were greater in the PTEC group than the Fresh group.

CONCLUSIONS: Despite a greater cancellation rate following PTEC, the overall ongoing pregnancy rates per retrieval and per transfer are greater than in fresh cycles. This supports the contention of a more receptive endometrium in cycles that are not subjected to ovarian stimulation.

TABLE 1.

	Fresh	Thaw	P-Value
Cycles	172	172	
Patient age	33.1 ± 4.3	33.1 ± 4.3	NS
2pn oocytes	11.9 ± 7.6	11.8 ± 7.5	NS
Day 3 FSH (IU/l)	7.1 ± 2.9	7.2 ± 2.5	NS
Blastocyst transfers	157	134	
Cancellation rate (%)	8.7	22.9	0.0009
Blastocysts transferred	316	261	
Mean # transferred	2.0 ± 0.5	1.9 ± 0.5	NS
Fetal heartbeats	111	142	
Implantation rate (%)	35.1	54.4	<0.0001
Pregnancy loss rate (%)	31.0	22.8	NS
Ongoing pregnancies	69	88	
Ongoing per transfer (%)	43.9	65.7	0.0002
Ongoing per retrieval	40.1	51.2	0.049