CONCLUSION: Out of the two studies using mouse zygotes, we may assume that the ICCP after VIT is, in contrary to the common beliefs, lower than the one observed after a SF procedure. Our data reinforce the concept that it is not justified to be sceptical about the use of high concentration of CPs for VIT.

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COMPARISON OF CLINICAL OUTCOMES BETWEEN SINGLE AND DOUBLE FROZEN-THAWED BLASTOCYST EMBRYO TRANSFER ACCORDING TO THE DAY OF VITRIFICATION. K. E. Lee,[a] S. M. Kang,[a] H. J. Jeong,[a] J. C. Kim,[a] S. G. Lee,[a] J. H. Lim.[b] [a]Maria Fertility Clinic, Daegu, Republic of Korea; [b]Maria Fertility Hospital, Seoul, Republic of Korea.

OBJECTIVE: To compare the efficacy of single frozen-thawed blastocyst embryo transfer (SFBT) versus double frozen-thawed blastocyst embryo transfer (DFBT) according to the day of vitrification.

DESIGN: Retrospective study.

MATERIALS AND METHODS: A total of 1427 women less than 37 years with their autologous SFBT cryopreserved on day 5 (5d-SFBT, n=691) or day 6 (6d-SFBT, n=237) and DFBT on day 5 (5d-DFBT, n=329) or day 6 (6d-DFBT, n=170) from January 2009 to December 2010 were included. The embryos reached the blastocyst stage on day 5 or 6 were vitrified using EM-grid following artificial shrinkage. Embryo thawing was conducted on day 4 after ovulation, and then surviving blastocysts were transferred into uterus cavity on day 5 after ovulation. Age (32.0±2.7 yrs vs. 32.2±2.8 yrs) and cause of infertility were not different in the SFBT group and the DFBT group. However, duration of cryopreservation (10.1±13.0 months vs. 13.0±14.5 months, $P<0.001$) was significantly shorter in the SFBT group compared with the DFBT group.

RESULTS: The clinical pregnancy (35.3% vs. 34.0%, $P=0.691$), ongoing pregnancy rates (28.8% vs. 28.9%, $P=0.980$) and were not significantly different between the 5d-SFBT group and the 5d-DFBT group. However, the clinical pregnancy (17.7% vs. 27.7%, $P=0.017$) and ongoing pregnancy rates (12.7% vs. 21.8%, $P=0.015$) were significantly lower in the 6d-SFBT group compared with the 6d-DFBT group. The implantation rate (35.6% vs. 21.1%, $P<0.001$) of the 5d-SFBT group was significantly higher than that of the 5d-DFBT group, while implantation rate (17.7% vs. 15.3%, $P=0.437$) of the 6d-SFBT group was similar to the 6d-DFBT group. Multiple pregnancy rates were significantly lower in the SFBT group compared with the DFBT group regardless of the day of vitrification.

CONCLUSION: This study showed that the SFBT developed on day 5 resulted in comparable clinical outcomes compared to DFBT while the SFBT formed on day 6 was a significantly lower clinical outcomes compared to DFBT.

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IMPORTANCE OF ENDOMETRIAL THICKNESS IN FROZEN-THAWED EMBRYO TRANSFER: DIFFERENCE BETWEEN HORMONE REPLACEMENT PROTOCOL AND NATURAL CYCLE PROTOCOL. I. H. Park,[a] K. H. Lee,[a] H. G. Sun,[a] S. K. Kim,[a] J. H. Lee,[a] G.-H. Jeon.[b] [a]Mamapapa & Baby OB&GY, Ulsan, Republic of Korea; [b]OB/GY, Inje University, Haeundae Paik Hospital, Busan, Republic of Korea.

OBJECTIVE: Endometrial (EM) thickness is an important prognostic factor of fresh embryo transfer and its significance has been proven by many studies. However, only few studies have evaluated the relationship between endometrial thickness and cycle outcome in frozen-thawed embryo transfer (FET). The purpose of this study is to assess the values of EM thickness in hormone replacement(HRT) cycle FET and in natural cycle FET.

DESIGN: A retrospective study.

MATERIALS AND METHODS: We retrospectively reviewed 487 FET cycles using HRT protocol and natural cycle protocol from November 2010 to November 2011. We divided into two groups by EM thickness. Thin EM group was defined by EM<9 mm and thick EM group was defined by EM ≥9mm. We compared cycle outcomes of the two groups in HRT protocol and in natural cycle protocol.

RESULTS: In each group, number of transferred embryos and embryo quality score reflected no difference. In natural cycle protocol, pregnancy rates of thin EM group and thick EM group were similar [31.1%(33/106) vs. 33.3%(52/156), $P=0.40$]. Ongoing pregnancy rates of thin EM group and thick EM group also were similar [29.2%(31/106) vs. 32.1%(50/156), $P=0.12$]. In HRT protocol, pregnancy rates of thick EM group were significantly higher than thin EM group [46.3%(50/108) vs. 34.2%(40/117), $P=0.04$]. Ongoing pregnancy rates of thick EM group were also significantly higher than thin EM group [40.7%(44/108) vs. 29.9%(35/117), $P=0.02$].

CONCLUSION: In HRT cycle FET, EM thickness above 9 mm on the day of P supplementation is associated with higher pregnancy rates compared with EM thickness below 9 mm. EM thickness can be used as a predictor of IVF prognosis in HRT cycle FET. However, in natural cycle FET, pregnancy rates did not show significant difference by EM thickness. Therefore, we should pay more attention to endometrial thickness in HRT cycle FET rather than in natural cycle FET.

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DOES ARTIFICIAL COLLPASE OF HUMAN DAY 6 BLASTOCYSTS PRIOR TO THE COOLING STEPS OF VITRIFICATION IMPROVE THEIR PROBABILITY OF INCREASED OUTCOME? J. Liebermann, E. J. Pelts, J. M. Matthews, S. R. Sanchez, J. Rapisarda, K. Lederer. Fertility Centers of Illinois, Chicago, IL.

OBJECTIVE: Today, vitrification of human blastocysts provides an excellent outcome. However, often there is a difference in outcome between day 5 and day 6 vitrified blastocysts. Day 6 blastocysts might have less ability to get sufficiently dehydrated prior to the vitrification steps. By collapsing the blastocoele prior to the vitrification procedure, it would allow sufficient dehydration of these cells and improve their implantation potential. Therefore, we evaluated the effectiveness of artificially collapsing day 6 blastocysts vitrified without artificial collapse to those that were artificially collapsed.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: A total of 627 frozen embryo transfers (FET; patient average age 35.4±4.9) using day 6 blastocysts without artificial collapse (group A) were analyzed. Group B contained a total of 65 FET (patient average age 35.4±5.2) with artificially collapsed day 6 blastocysts. Prior to vitrification, the junction between two trophectoderm cells in the blastocyst was located and one laser shot at 100% power with a 500ms pulse (Hamilton Thorne) was released. For vitrification, the High Security Vitrification Kit (CryoBioSystem, France) was used as a closed carrier, with a mixture of 15% Ethylene Glycol/Dimethyl Sulfoxide (v/v)+0.5M sucrose. Statistical significance was evaluated by Chi-Square.

RESULTS: Group A and B didn't show any statistical significance for the average number of embryos transferred (1.8 vs. 1.7) and survival rate (98.1 vs. 98.7%). There was a significant increase in the clinical pregnancy and implantation rate in group B compared with group A (57.5% vs. 39.9%; $P<.001$; and 44.6% vs. 28.4%; $P<.001$). The overall ongoing pregnancy was significant higher in group B compared with group A (51.7% vs. 35.1%; $P<.001$).

CONCLUSION: Our data clearly reveals the benefit of artificial collapse of day 6 blastocysts prior to the steps of vitrification by improving their probability of increased pregnancy rate and implantation potential.

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COMPARISON OF TWO DIFFERENT PROTOCOLS FOR THE CRYOPRESERVATION OF TESTICULAR TISSUE IN RATS. M. R. M. Radaelli,[a] C. G. Almodin,[a] P. M. Almodin,[a] V. C. M. Camara,[a] H. Meisler,[b] A. J. Gonçalves.[c] [a]Human Reproduction, Materbaby Reprodução Humana, Maringá, PR, Brazil; [b]Clinical Pathology, Lapam - Laboratorio de Patologia de Maringá, Maringá, PR, Brazil; [c]Urology, Santa Casa de Misericórdia de São Paulo, SP, Brazil.

OBJECTIVE: The ideal protocol for the cryopreservation of testicular tissue remains to be found, which has stimulated several lines of research. The objective of this study was to compare two different protocols for the cryopreservation of testicular tissue in rats.

DESIGN: Testicular tissue from immature rats were cryopreserved using two different freezing protocols, thawed, histologically assessed and compared to samples of fresh tissue.

MATERIALS AND METHODS: A total of 10 testicles were obtained from five sexually immature Wistar rats, which were submitted to bilateral orchiectomy under anesthesia. After removing the tunica albuginea, a sample of fresh testicular tissue was removed from each testicle and immediately

sent to histological processing and analysis (control group), while the remaining tissue samples were cryopreserved using ultrarapid (vitrification) or slow-programmed freezing protocols. After thawing, test samples received the same histological treatment as the control group, when damage to the seminiferous tubules was semi-quantitatively determined according to the score described by Milazzo et. al. (2008). After each plate was scored, the mean value of the sum of all scores was determined and the three groups were statistically compared using Mann Whitney's test.

RESULTS: The mean value for the sum of scores for both the vitrification and slow-programmed freezing protocols was 0.2, while for the control group it was 0. No statistically significant differences were found among groups, indicating that cryopreserved testicular tissue structure was histologically preserved after thawing.

CONCLUSION: Both the slow-programmed freezing and the vitrification protocols showed equivalent results in histological terms; the next step being the investigation of the functionality of testicular cells.

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TWICE-FROZEN EMBRYOS HAVE IMPLANTATION POTENTIAL SIMILAR TO ONCE-FROZEN EMBRYOS. S. T. Daneshmand,[a,b] B. S. Shapiro,[a,b] F. C. Garner,[a,b] M. Aguirre,[a] C. Hudson.[a] [a]Fertility Center of Las Vegas, Las Vegas, NV; [b]Dept of Obstetrics and Gynecology, University of Nevada School of Medicine, Las Vegas, NV.

OBJECTIVE: Compare implantation potential of autologous thawed blastocysts derived from fresh or thawed bipronuclear oocytes.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: This study included all autologous blastocyst thaw cycles in the period 2006-2011. Patients with fresh or slow-frozen bipronuclear oocytes had their cohorts, whether fresh or thawed, cultured to the blastocyst stage before transfer, regardless of cohort size. Supernumerary blastocysts were conventionally slow-frozen. Patients seeking another chance at pregnancy had supernumerary blastocyst(s) thawed for transfer, and the efficacy of those thawed blastocysts are compared here. Ongoing pregnancy was defined by viable fetal cardiac activity at 10 weeks gestation. Implantation rate was the ratio of the number of observed fetal hearts to the number of transferred blastocysts. Fisher's exact test was used to compare nominal outcomes. Wilcoxon's test was used to compare numeric measures.

RESULTS: Results are provided in Table 1. The two groups did not differ significantly in age, survival rate, implantation rate, or ongoing pregnancy rate per transfer. The group using twice-frozen embryos had significantly fewer transferred blastocysts.

CONCLUSION: Frozen-thawed blastocysts derived from thawed bipronuclear oocytes have implantation potential similar to that of frozen-thawed blastocysts derived from fresh bipronuclear oocytes. The use of these twice-frozen twice-thawed embryos provided a reasonable chance of success in patients who underwent dedicated freeze-thaw cycles and later sought additional attempts at pregnancy.

TABLE 1.

	Thawed once	Thawed twice	*P*-value
Transfers	598	72	
Age (yr)	33.5±4.4	33.9±4.4	NS
Age range	22-44	23-43	
Survival (%)	90.8	88.9	NS
Transferred blastocysts	1.85±0.58	1.60±0.57	0.0003
Ongoing pregnancies	258	27	
Ongoing/transfer (%)	43.1	37.5	NS
Fetal hearts	361	39	
Implantation rate (%)	32.6	33.9	NS

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CRYOPRESERVATION OF OOCYTES: OUTCOME OF 2409 FREEZING CYCLES. E. Porcu, L. Notarangelo, F. Fabbri, G. Damiano, C. Zacà, L. Cipriani. IVF Center, Sant'orsola Malpighi Hospital University, Bologna, Italy.

OBJECTIVE: To establish the efficiency of oocyte cryopreservation in a large number of cycles.

DESIGN: Prospective longitudinal study at the University of Bologna.

MATERIALS AND METHODS: From 1996 to 2012 three thousand patients undergoing assisted reproduction have been enrolled in our oocyte cryopreservation program. After induction of superovulation and oocyte retrieval, excess oocytes were stored with slow freezing using propandiol 0.1 M and sucrose 0.2-0.3 M. After rapid thawing, survived oocytes were inseminated with ICSI (Intracytoplasmatic Sperm Injection).

RESULTS: In 2409 treatment cycles, 19061 MII oocytes were frozemn. In 2235 thawing cycles, 11338 oocytes were thawed. The surgical rate was 70%. 2pn fertilization rate was 68% with subsequent 90 cleavage rate. 458 pregnancies were achieved (24% per transfer, 20.5% per thawing cycle) with the delivery of 357 children. Two children had minor malformation (0.6%). The oldest children are now 15 years old displaying normal growth and pubertal development.

CONCLUSION: The cryostorage of human female gametes has several indications. First of all, oocytes cryopreservation might replace embryo freezing. Embryo cryopreservation often only postpones the problem of excess embryos and raises legal, ethical, moral and religious reactions. The alternative of oocytes storage is devoted of ethical and moral problems. Further, egg freezing can be a fertility preservation choice. Oocyte storage may also contribute to increase in vitro fertilization routine flexibility and to reduce the risk of developing ovarian hyperstimulation syndrome postponing oocyte insemination and embryo transfer. But is human oocyte cryopreservation ready for routine clinical application? Surprisingly, human oocyte cryopreservation is still relegated in the research area and is regarded as an experimental technique. The present study support the efficiency and safety of oocyte cryopreservation in a large number of cases.

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THE LATE CLEAVED EMBRYOS, FROZEN CYCLE IS BETTER THAN FRESH CYCLE. J. H. Lee, K. H. Lee, I. H. Park, H.-G. Sun, S. G. Kim, Y. Y. Kim. Infertility Lab, Mamapapa & Baby OB&GY, Ulsan, Republic of Korea.

OBJECTIVE: In general, the late cleaved-embryos have low implantation potential in fresh embryo transfer cycles, may be due to embryo-endometrium asynchrony. However, FETs seems to improve embryo-endometirium synchrony by control of thawing day. The aim of this study is to compare the implantation potential of the late cleaved-embryos ($\leq$6 cell) on day 3 in frozen thawed embryo transfers and Fresh embryo transfers.

DESIGN: Retrospective study.

MATERIALS AND METHODS: We analyzed 468 cycles of the late cleaved embryo from January 2010 to January 2012. The patient was divided by two groups : group A (n=271), Fresh late cleaved embryo trnasfer cycels, group B (n=197), Frozen-thawed late cleaved embryo transfer cycles. Cryopreserved embryos were transferred to patients either in natural cycle or in a hormonally manipulated artificial cycle. Embryos were frozen on day 3 using a vitrification protocol. Late cleaved embryos were thawed on 1 day before ET and we selected 8-cell embryos developed well on the next day. FETs were performed by same physician on day 3. We compared clinical pregnancy rate between two groups.

RESULTS: There were no differences in patient age (A vs. B, 36.7±4.0 vs.35±4.1), the mean number of transferred embryos (2.1±1.2 vs. 2.2±1.3) and endometrial thickness (10.2±1.6 vs. 10.1±2.0) and embryo quality between two groups. However, the clinical pregnancy rates were significantly higher in group B (34.3%) than group A (26.9%, $P<0.01$).

CONCLUSION: In our study, the pregnancy rates were significantly higher in frozen-thawed late cleaved embryo transfers than fresh embryo transfers. In our results, low implantation potential of the late cleaved embryos can be overcome by thawing 1 day before ET, in FETs. It seems that the thawing 1 day before ET of late cleaved embryos enabled more appropriate synchronization between embryonic and endometrial development. Therefore the late cleaved embryo cryopreservation may be an effective alternative to fresh embryo transfer.

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ALTERATION OF HIGH-POLARIZED MITOCHONDRIA DISTRIBUTION BY VITRIFICATION OF MOUSE IMMATURE OOCYTE MAY AFFECT EMBRYONIC DEVELOPMENT. S. Y. Yoon, S. K. Cha, N. J. Yang, J. H. Eum, W. S. Lee, D. R. Lee. Fertility Center of CHA Gangnam Medical Center, CHA University, College of Medicine, Seoul, Korea.

OBJECTIVE: Mitochondria have a central role for cell viability and in early mammalian embryogenesis. Asymmetric distribution of mitochondria at pronuclear stage has been associated with asymmetric segregation into different