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ASSISTED REPRODUCTION TECHNOLOGIES

Comparison of the clinical outcomes between fresh blastocyst and vitrified-thawed blastocyst transfer

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Abstract
Objective To compare the clinical outcomes between fresh and vitrified-thawed day 5 blastocyst transfers.
Design Retrospective case control study.
Setting Tertiary referral center.
Patient(s) Patients 38 years of age or less who underwent IVF/ICSI cycles with fresh or frozen-thawed blastocysts transferred from June 1, 2009 to November 30, 2011
Intervention(s) Vitrification and thawing of day 5 blastocysts using the Cryotop method. (Kitazato BioPharma Co., Ltd., Fuji city, Shizuoka, Japan)
Main outcome measure(s) Clinical pregnancy rate, implantation rate, ongoing pregnancy rate, and multiple pregnancy rates.
Results Of the 118 cycles in the fresh transfer group, 234 blastocysts were transferred. The clinical pregnancy rate was 66.1 % and implantation rate was 50.9 %. The ongoing pregnancy rate was 56.8 % and the rates for singleton and twin pregnancies were 53.7 % and 44.8 %. Of the 59 cycles in the vitrified-thawed group, 111 blastocysts were transferred. The clinical pregnancy rate was 59.3 % and implantation rate was 43.2 %. The ongoing pregnancy rate was 47.5 % and the rates for singleton and twin pregnancies were 60.7 % and 39.3 %. The clinical pregnancy rate, implantation rate and ongoing pregnancy rate did not differ significantly between the two groups.
Conclusions The implantation rates were not significantly different between the fresh and the vitrified-thawed groups. Thus, single embryo transfer may be considered in fresh cycles to decrease multiple pregnancy rates. The surplus embryos should be vitrified for the frozen embryo transfer to improve the cumulative pregnancy rate.

Keywords Vitrification · Blastocyst · Implantation · Pregnancy

Pei-Yun Ku and Robert Kuo-Kuang Lee contributed equally to this work.

Capsule Vitrified blastocysts have similar implantation potential in comparison to fresh blastocysts.

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Introduction

Blastocyst transfers in IVF protocols led to an increase in implantation rates, higher pregnancy rates, and a decrease of high-order multiple gestations resulting from a reduction in the number of embryos transferred compared with embryos transferred at the cleavage stage [1]. Thus, the idea of cryopreservation of the superfluous embryos evolved. There are two basic techniques currently applied in embryo cryopreservation: the slow-freezing technique and the vitrification technique. Vitrification is thought to be safer and more cost effective than slow freezing. During the rapid freezing process with high concentration of cryoprotectants, less intracellular ice crystal formation reduces cellular damage and results in better reproductive potential after thawing [2, 3].

Though, the procedures of freezing and thawing have some adverse effects on the embryos, there are significantly higher ongoing pregnancy, clinical pregnancy and implantation rates in cycles with vitrified-thawed day 2 embryos than in cycles with fresh day 2 embryos [4]. The cycles of thawed bipronuclear (2PN) oocytes subjected to post-thaw extended culture to the blastocyst stage also resulted in a higher clinical pregnancy rate than did fresh embryo transfer cycles [5]. The vitrified blastocysts retained good developmental competency after thawing. High pregnancy and implantation rates can be expected once the blastocysts go through vitrification and thawing because they have the potential to develop as fresh blastocysts [6].

The developmental potential of cryo-thawed blastocysts is a concern due to synchronization of endometrial receptivity. Natural endometrial preparation is superior to hCG and hormonally manipulated endometrial preparation [3, 7]. Better synchronization between the embryo and endometrium is believed in frozen-thawed natural cycles than in stimulated cycles [4, 5].

The objective of this study was to compare the clinical outcomes between fresh and vitrified-thawed day 5 blastocyst transfers with the same morphologic quality of embryos.

Methods

An institutional review board approved this study before initiation. The registration number is 11MMHIS081. The study is a retrospective case control study to review the IVF/ICSI cycles from June 1, 2009 to November 30, 2011 at Mackay Memorial Hospital. It included 118 cycles of fresh blastocyst transfer and 59 cycles of vitrified-thawed blastocyst transfer. The inclusion criteria were age, 38 years of younger and good-quality blastocyts (morphology better than or equal to 3BB, according to Gardner's criteria). The exclusion criteria were fresh or vitrified day 6 blastocysts and post-thaw extended culture from day 3 embryo to day 5 blastocyst.

Our vitrification and thawing protocols were applied from the Cryotop safety kit and modified from the Cryotop method. (Kitazato BioPharma Co., Ltd., Fuji city, Shizuoka, Japan).

Pregnancy definition

Clinical pregnancy which was defined as ICMART and WHO revised glossary of ART terminology was proven by the appearance of a gestational sac in the ultrasound examination [8]. The ongoing pregnancy was defined as pregnancy beyond 12 gestational weeks. Abortion was defined as any clinical pregnancies that did not continue to the point of an ongoing pregnancy.

Statistical analysis

The main outcome measures were compared between the groups using the Chi-square test or Fisher exact test for comparison of percentages and Student's t test for comparison of mean values. Statistical analyses were performed with PASW statistical software, version 18. $P<0.05$ was considered significant for all measures.

Results

Of the 145 cycles in the fresh blastocyst transfer group, 27 cycles were abandoned due to poor quality blastocysts and of the 156 cycles in the vitrified-thawed blastocyst group, 97 cycles were excluded due to poor quality blastocysts, vitrified on Day 3 or Day 6. The characteristics of patients in the two groups are listed in Table 1. There was no significant difference in the proportion of ICSI between the two groups. Of the 118 cycles in the fresh group, 234 blastocysts were transferred (mean number of embryos transferred per cycle: 1.98±0.130). The clinical pregnancy rate was 66.1 % and the implantation rate was 50.9 %. The ongoing pregnancy rate was 56.8 % and the rates of singleton and twin pregnancies were 53.7 % and 44.8 %, respectively. Of the 59 cycles in the vitrified-thawed blastocyst group, 111 blastocysts were transferred (mean number of embryos transferred per cycle: 1.88± 0.326). The clinical pregnancy rate was 59.3 % and the implantation rate was 43.2 %. The ongoing pregnancy rate was 47.5 % and the rates of singleton and twin pregnancies were 60.7 % vs. 39.3 % (Table 2).

Discussion

There was no significant difference in clinical pregnancy rates, implantation rates, or ongoing pregnancy rate between the fresh blastocyst transfer group and the vitrified-thawed

Table 1 Patient characteristics by type of blastocyst transfers

	Fresh group	Vitrified group	P value
No. of cycles	118	59	
Age	32.24±2.99	33.36±3.02	0.020
ICSI (%)	64 (54.2)	35 (59.3)	0.523
Infertility factor (%)			
Tubal factor	31 (26.3)	10 (16.9)	0.166
Male factor	46 (39.0)	23 (39.0)	> 0.999
Endometriosis	16 (13.6)	5(8.5)	0.324
Ovulation dysfunction	10 (8.5)	7(11.9)	0.471
Unexplained	5 (4.2)	9 (15.3)	0.016
Multiple factor	10 (8.5)	5 (8.5)	> 0.999

Values are presented as n (%) or mean±SD

$P<0.05$ is defined as significantly different

blastocyst transfer group. This data is meaningful to us because all vitrified blastocysts were surplus after fresh embryo transfer cycles. To achieve a better live birth rate, the selection methods call for choosing the best fresh embryos for transfer. Thus, the remaining embryos that survive the freezing and thawing procedures supposedly have a reduced chance of implantation. Nevertheless, the implantation and pregnancy rates were not reduced in the vitrified-thawed group compared with the fresh blastocyst transfer group in this investigation.

To explain the similar outcomes between the fresh and thawed embryo transfers, multiple factors may be considered. First, the improved techniques of vitrification results in better survival and developmental potential after thawing. Kuc et al. reported that the clinical pregnancy rates of the vitrification group and slow-freezing group of day 5 or day 6 blastocysts were significantly different. The clinical pregnancy rates of the vitrification and slow-freezing groups were 50.4 % and 25.9 % ($P<0.05$), respectively [9]. Due to the high concentration of cryoprotectant and fast cooling rate, the vitrification process avoids intracellular ice crystal formation during cooling and prevents injury to the cryopreserved cells. Additionally, it protects the cells from damage due to ice crystals during thawing [2].

Initially, we were not sure whether the high concentration of cryoprotectant was chemically toxic to the cryopreserved cells. Since the vitrification procedure of murine embryos was first presented in 1985, the protocol and solutions have been modified to be less toxic and more efficient [10]. Hong et al. reported improved clinical results between two cohorts in the same laboratory. They modified the protocol due to the finding that the permeation of the cryoprotectant into the embryo was faster at a higher temperature [6]. Minimum vitrification solution is used in Cryotop method. This approach increases the cooling and warming rates, which improve post-thaw survival and development of the embryo. Larman et al. analyzed gene expression of cryopreserved mouse blastocysts using microarrays. The gene expressions, including involvement of protein metabolism, transcription, cellular biosynthesis, and development were only significantly influenced by slow-freezing. There was no significantly influenced gene expression in non-cryopreserved or vitrification groups [11].

Endometrial receptivity and synchronization between the embryo and endometrium are very important in cryopreserved-thawed embryo transfer cycles and in fresh embryo transfer cycles, regardless of the cleavage stage, post-thaw extended culture, or blastocyst stage. Cryopreserved-thawed embryos transferred in a natural ovulatory cycle resulted in better clinical outcomes than fresh embryos transferred in stimulated cycles [3–5, 7, 12]. Gene expression, various cytokine signaling pathways, the complement and coagulation factor cascade, and leukocyte transendothelial migration are different in natural and stimulated cycles [4]. Haouzi et al. reported that the TGFβ signaling, leukocyte transendothelial migration, and the cell cycle of the endometrium in stimulated cycles were defective [13]. The same laboratory also analyzed the gene expression of endometrium between the natural cycle and stimulated cycles, including the GnRH agonist long protocol and GnRH antagonist protocols by DNA microarray analysis. There were significantly different expression patterns of endometrial chemokines and growth factors between stimulated cycles in comparison with natural cycles. Especially, the differences

Table 2 Clinical outcomes of fresh blastocyst and vitrified-thawed blastocyst transfers

	Fresh group	Vitrified group	P value
No. of cycles	118	59	
No. of blastocysts transferred	1.98±0.130	1.88±0.326	0.004
Clinical pregnancy	78 (66.1)	35 (59.3)	0.376
Implantation	118 (50.9)	48 (43.2)	0.228
Abortion	11 (14.1)	7 (20.0)	0.428
Ongoing pregnancy	67 (56.8)	28 (47.5)	0.241
Singleton pregnancy	36 (53.7)	17 (60.7)	0.532
Twin pregnancy	30 (44.8)	11 (39.3)	0.622
Triplet pregnancy	1 (1.5)	0	> 0.999

Values are presented as n (%) or mean±SD

$P<0.05$ is defined as significantly different

were more obvious in GnRH agonist long protocol than in the GnRH antagonist protocols. Among the down-regulated genes specific to the GnRH agonist protocol, numerous genes play important roles in cell-cycle function and more especially in checkpoint regulation [14].

Zhu et al. reported higher pregnancy and implantation rates in vitrified-thawed blastocysts than in fresh blastocyst transfer cycles. The clinical pregnancy rate of fresh and vitrified blastocyst transfer groups were 36.4 % and 55.1 %, respectively ($P<0.05$) and the implantation rate of the fresh and vitrified group was 25.2 % and 37.0 % ($P<0.05$) [12]. We also had similar clinical pregnancy and implantation rates (59.3 % and 43.2 %, respectively) in the vitrified-thawed blastocyst transfer group.

In our current study, the implantation rate, which reflects the endometrial synchronization and receptivity between the blastocyst and endometrium, was 50.9 % in the fresh blastocyst transfer group and 43.2 % in the vitrified-thawed group; they did not differ significantly. Thus, single embryo transfer may be considered in fresh cycles to decrease multiple pregnancy rates, if blastocysts of good quality are obtained. The surplus embryos would be vitrified for frozen embryo transfer to improve the cumulative pregnancy rate. In conclusion, as long as results are reliable for vitrified-thawed cycles, all available blastocysts can be vitrified in patients for whom fresh blastocyst transfer is unsuitable, such as patients at risk of OHSS, those with a history of repeat failed fresh embryo transfers, and those in need for preimplantation genomic diagnosis.

References

1. Gardner DK, Balaban B. Choosing between day 3 and day 5 embryo transfers. Clin Obstet Gynecol. 2006;49:85–92.
2. Kuleshova LL, Lopata A. Vitrification can be more favorable than slow cooling. Fertil Steril. 2002;78:449–54.
3. Chang EM, Han JE, Kim YS, et al. Use of the natural cycle and vitrification thawed blastocyst transfer results in better in-vitro fertilization outcomes. J Assist Reprod Genet. 2011;28:369–74.
4. Aflatoonian A, Oskouian H, Ahmadi S, Oskouian L. Can fresh embryo transfers be replaced by cryopreserved-thawed embryo transfers in assisted reproductive cycles? A randomized controlled trial. J Assist Reprod Genet. 2010;27:357–63.
5. Shapiro BS, Daneshmand ST, et al. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen–thawed embryo transfer in normal responders. Fertil Steril. 2011;96:344–8.
6. Hong SW, Sepilian V, Chung HM, Kim TJ. Cryopreserved human blastocysts after vitrification result in excellent implantation and clinical pregnancy rates. Fertil Steril. 2009;92:2062–4.
7. Fatemi HM, Kyrou D, Bourgain C, et al. Cryopreserved-thawed human embryo transfer: spontaneous natural cycle is superior to human chorionic gonadotropin–induced natural cycle. Fertil Steril. 2010;94:2054–8.
8. Zegers-Hochschild F, et al. International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009. Fertil Steril. 2009;92:1520–4.
9. Kuc P, Kuczyñska A, et al. Vitrification vs. slow cooling protocol using embryos cryopreserved in the 5th or 6th day after oocyte retrieval and IVF outcomes. Folia Histochem Cytobiol. 2010;48:84–8.
10. Rall WF, Fahy GM. Ice-free cryopreservation of mouse embryos at −196 °C by vitrification. Nature. 1985;313:573–5.
11. Larman MG, Katz-Jaffe MG, et al. Analysis of global gene expression following mouse blastocyst cryopreservation. Hum Reprod. 2011;10:2672–80.
12. Zhu D, Zhang J, Cao S, et al. Vitrified-warmed blastocyst transfer cycles yield higher pregnancy and implantation rates compared with fresh blastocyst transfer cycles—time for a new embryo transfer strategy? Fertil Steril. 2011;95:1691–5.
13. Haouzi D, Assou S, Mahmoud K, et al. Gene expression profile of human endometrial receptivity: comparison between natural and stimulated cycles for the same patients. Hum Reprod. 2009;24:1436–45.
14. Haouzi D, Assou S, Dechanet C, et al. Controlled ovarian hyperstimulation for in vitro fertilization alters endometrial receptivity in humans: protocol effects. Biol Reprod. 2010;82:679–86.