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Research Article

Vitrified-Warmed Blastocyst Score Effects Pregnancy Outcomes: Towards a Single Blastocyst Vitrification and Transfer

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Abstract

No a referable quality selection criteria for a single vitrified-warmed blastocyst transfer. Therefore, the present study aimed to investigate the relationship between vitrified-warmed blastocyst score and clinical pregnancy outcomes. This retrospective analysis consisted of 221 patients undergoing two blastocysts transfer on thawing day. Implantation rate, fetal heart pregnancy rate, live-birth rate, multiple birth rate were analyzed.

When a patient received two high-quality vitrified-warmed blastocysts (≥3BB), implantation rate and fetal heart pregnancy rate were 48.2% and 65.5%, respectively. The multiple birth rates in this group were 44.4%. When two vitrified-warmed blastocysts (one ≥3BB and another <3BB) were available for transfer, implantation rate and fetal heart pregnancy rate were 34.5% and 52.7%. The multiple birth rate for this group was 29.6%. When only two general-quality vitrified-warmed blastocysts (<3BB) were transferred, implantation rate and fetal heart pregnancy rate were 21.7% and 35.9%, and the multiple birth rate was 21.7%.

The ability to transfer one good-quality vitrified-warmed blastocyst (≥3BB) should lead to fetal heart pregnancy rates greater than 52% and live birth rates greater than 36%. Results of the present study can provide guidelines for a single vitrified-warmed blastocyst transfer, which is an effective means of eliminating multiple gestations and avoiding the complications associated with such pregnancies.

Keywords: Vitrified-warmed blastocyst; Quality; Pregnancy outcome; Single-embryo transfer

Introduction

Multiple pregnancies are a complication of human Assisted Reproductive Technology (ART), rather than a successful result [1]. So, how to reduce the multiple pregnancy rates and maintain the acceptable overall live-birth rate have become research hotspots in the area of reproductive medicine. The strategy of a single embryo transfer is the most effective way to achieve single pregnancy.

Transfer of an embryo with a high potential for development and implantation is a key success factor in ART [2]. Routine blastocyst culture using sequential culture media *in vitro* can make a better assessment for embryo viability and may confer a selection advantage [3]. Moreover, blastocyst transfer has been associated with a higher implantation rate [4-7] and a better synchronization between endometrial receptivity and embryo [8].

In a conventional *In Vitro* Fertilization (IVF) cycle, the use of exogenous gonadotropins results in Ovarian Hyper Stimulation Syndrome (OHSS). Cryopreservation programs are essential for patients who suffered from OHSS. Moreover, for infertile women with the polycystic ovary syndrome, frozen-embryo transfer was associated with a higher rate of live birth and a lower risk of the OHSS than fresh-embryo transfer after the first transfer [9], because vitrified-warmed blastocyst transfer occurs within a uterine environment that more closely resembles spontaneous conception [10]. In addition, cryopreservation process is used for the storage of supernumery embryos to increase cumulative pregnancy rate.

Transfer of two or more embryos may increase the chance of pregnancy for a patient, but also lead to multiple pregnancy, and maternal and neonatal complications. Therefore, many ART clinics are now opting for a single embryo transfer protocol that aims to eliminate the complications associated with multiple pregnancies [11,12]. To implement a single vitrified-warmed blastocyst transfer protocol successfully, it is necessary to determine the quality selection criteria of a blastocyst. Here, we report a retrospective analysis of patients receiving vitrified-warmed blastocysts transfer and the correlation of implantation rate and pregnancy outcome with respect to the blastocyst score.

Materials and Methods

Study population

Two hundred and twenty-one patients who had received vitrified-warmed blastocyst transfer between January 2013 and July 2015 were included in the analysis. Inclusive criteria were [1] two vitrified-warmed blastocysts were transferred on the thawing day, [2]

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Table 1: Patient demographics for vitrified-warmed blastocyst transfers.

		Patient group	
	Group 1	**Group 2**	**Group 3**
	(two blastocysts ≥3BB)	(one blastocyst ≥3BB)	(two blastocysts <3BB)
		(one blastocyst <3BB)	
No. of transfers	55	74	92
*Cycle number	1.22 ± 0.4	1.20 ± 0.4	1.24 ± 0.5
*Mean age (y)	31.0 ± 5.0	31.4 ± 4.1	32.4 ± 4.3
Age range	20–42	22–40	23–43
*Duration of infertility (y)	4.3	4.6	5.1
*FSH	7.39 ± 1.56	7.71 ± 3.03	7.49 ± 1.84
*LH	6.36 ± 4.75	5.40 ± 2.73	5.54 ± 3.42
The number of retrieved oocytes	22.35 ± 10.67[a]	18.35 ± 9.06	17.83 ± 8.19
Fertilization			
IVF	47	54	60
ICSI	5	11	19
Half-ICSI	3	9	13
Indications			
Male factor only	5 (9.1%)	8 (10.8%)	17 (18.5%)
Female factor only	41 (74.5%)	54 (73.0%)	58 (63.0%)
Tubular factor	24	41	43
Endometriosis	1	0	2
Ovarian factor	5	4	3
Combined	11	9	10
Combined male/female	6 (10.9%)	10 (13.5%)	13 (14.1%)
Unexplained	3 (5.5%)	2 (2.7%)	4 (4.4%)
*NC *vs.* HRT	34.5% *vs.* 65.5%	52.7% *vs.* 47.3%	43.5% *vs.* 56.5%

*No significant differences among three groups.
[a]*P*<.01 (Group 1 *vs.* Group 2; Group 1 *vs.* Group 3).

the vitrified-warmed blastocyst transfer was not a part of sequential transfer, [3] the endometrial preparation programs were natural cycles or hormone replacement therapy cycles. Institutional Review Board approval was not obtained because the study was not a prospective but a retrospective analysis of case results. The procedures used in this retrospective cohort study were in accordance with the guidelines of the Helsinki Declaration on Human Experimentation and Good Clinical Practice (CGP). The informed consent requirement was exempted because we only retrospectively accessed a de-identified database for analytical purposes.

Embryo vitrification and warming

After shrinkage of the blastocoel, embryos were transferred into equilibration solution (KITAZATO, Tokyo, Japan) for 8 min at room temperature. After they were washed in vitrification solution for three times, blastocysts were picked up in an extremely small volume (<0.1μL) of vitrification solution, transferred onto the open pulled straw and placed into liquid nitrogen rapidly. Subsequently, a plastic tubule was covered the straw to provide protection during storage in liquid nitrogen.

For blastocyst thawing, the plastic tubule was removed from the straw (while the straw was immersed in liquid nitrogen), and embryos on the straw was placed directly into 0.5 mL of preheating thawing solution (KITAZATO, Tokyo, Japan) for 1 minute. The blastocysts were subsequently transferred into 500 μL of diluent solution two for 3 minutes at thermal platform and then into 500 μL of washing solution one and two at thermal platform for 5 minutes, respectively. After warming, all embryos were cultured in pre-equilibrated G2 Plus (Vitrolife, Göteborg, Sweden) and allowed to recover for 1-2 h, at which stage a final assessment was made to determine whether the embryos were transferred or not; a blastocyst with more than 50% intacted-cells and some re-expansion blastocoel cavity was considered survived [13].

Embryo assessment

A three-part scoring system developed by Gardner and Schoolcraft [14] was used to grade human blastocyst. Briefly, blastocysts were scored according to their expansive degree and hatching status, as below: 1, an early blastocyst with a blastocoels cavity which is less than half of the embryo volume; 2, a blastocyst with a blastocoels that is half of or larger than half of the embryo volume; 3, a blastocyst with a blastocoel that is full of the whole embryo; 4, an expanded blastocyst with a blastocoel filling the embryo and a thinning zona pellucida; 5, a hatching blastocyst with the trophectoderm beginning

Table 2: Effect of vitrified-warmed blastocyst score on clinical pregnancy outcome.

		Patient group	
	Group 1	**Group 2**	**Group 3**
	(two blastocysts ≥3BB)	(one blastocyst ≥3BB)	(two blastocysts <3BB)
Variable		(one blastocyst <3BB)	
No. of transfers	55	74	92
No. of embryos transferred	2	2	2
Endometrial thickness	9.79 ± 2.89	9.54 ± 1.91	9.67 ± 2.27
Implantation rate [a]	53/110(48.2%)	51/148(34.5%)	40/184(21.7%)
Pregnancy rate			
Biochemical	38/55(69.1%)[b]	45/74(60.8%)[b]	38/92(41.3%)
Fetal heart	36/55(65.5%)[c]	39/74(52.7%)[c]	33/92(35.9%)
Birth rate			
Live	27/55(49.1%)[d]	27/74(36.5%)[de]	23/92(25.0%)[e]
Multiple	12/27(44.4%)	8/27(29.6%)	5/23(21.7%)
Miscarriage rate			
Subclinical	2/38(5.3%)	6/45(13.3%)	5/38(13.2%)
Clinical	9/36(25.0%)	12/39(30.8%)	10/33(30.3%)

[a] *P*<.05 (significant linear trend among all groups).
[b] *P*<.05 (Group 1 *vs.* Group 3; Group 2 *vs.* Group 3).
[c] *P*<.05 (Group 1 *vs.* Group 3; Group 2 *vs.* Group 3).
[de] *P*<.01 (Group 1 *vs.* Group 3).

Table 3: Neonatal outcomes for vitrified-warmed blastocyst transfers.

		Patient group	
	Group 1	**Group 2**	**Group 3**
	(two blastocysts ≥3BB)	(one blastocyst ≥3BB)	(two blastocysts <3BB)
		(one blastocyst <3BB)	
Gestational age [a] (wk)	37.0 ± 2.9	37.4 ± 2.5	37.3 ± 3.0
Preterm birth rate	10/27 (37.0%)	7/27 (25.9%)	4/23 (17.4%)
Live-birth weight [a] (g)			
singleton	3004 ± 710*	3251 ± 452**	3079 ± 414**
twins	2452 ± 563	2216 ± 420	2494 ± 331
Low-birth-weight rate			
singleton	3/14 (21.4%)	1/18 (5.6%)**	1/15 (6.7%)*
twins	8/22 (36.4%)[b]	11/16 (68.8%)[c]	4/8 (50.0%)[bc]
Neonatal death rate	0	0	1/23 (4.3%)

[a] Mean ± standard deviation.
Live-birth weight a (g):
* *P* <.05 (Group 1: singleton *vs.* twins)
** *P* <.01 (Group 2: singleton *vs.* twins; Group 3: singleton *vs.* twins)
Low-birth-weight rate:
** *P* <.001 (Group 2: singleton *vs.* twins)
* *P* <.05 (Group 3: singleton *vs.* twins)
[bc] *P* <.05 (Group 1 *vs.* Group 2)

to extrude from the zona pellucida; and 6, a hatched blastocyst which has completely escaped from the zona pellucida.

In addition, according to the development condition of inner cell mass and trophectoderm, the blastocysts graded as 3–6 was assessed as follows: for the inner cell mass: A, numerous cells packed tightly; B, several cells loosely grouped; or C, only very few cells; for the trophectoderm: A, large numbers of cells forming a cohesive epithelium; B, few cells forming a loose epithelium; or C, very few large cells.

Embryo transfer

Two vitrified-warmed blastocysts were transferred to patients using a Wallace catheter (Edwards-Wallace catheter; Marlow Technologies, Inc., Willoughby, OH) with ultrasonographic guidance.

Outcomes measured

Maternal age (years) was calculated at the time of vitrified-warmed embryo transfer. Blastocyst score was used as a measure of embryo quality and was determined by the scoring system. Biochemical pregnancy was assessed by measuring the β-hCG level in circulating blood at 14 days after embryo transfer; 75 mIU/mL or greater β-hCG was considered to be positive. The biochemical pregnancy rate was calculated by the β-hCG-positive pregnancies per embryo transfer cycle.

Biochemically pregnant patients were subsequently evaluated for the presence of fetal heart motion at 2 to 3 weeks using transvaginal ultrasonography. The implantation rate was calculated by fetal heart positive pregnancies per embryo transfer. The fetal heart pregnancy rate was calculated by fetal heart positive pregnancies per embryo transfer cycle. The live-birth rate was calculated by birthing events per embryo transfer cycle (where the birth outcome was known). The subclinical miscarriage rate was calculated by fetal heart negative pregnancies per β-hCG-positive pregnancies. The clinical miscarriage rate was calculated by fetal heart positive pregnancies that did not result in a live birth per fetal heart positive pregnancies (where the birth outcome was known). The multiple birth rate was calculated by the number of twin births per total birth events (where the birth outcome was known). Gestational age was calculated by determining the number of days between embryo transfer and end of pregnancy plus 19 days. The preterm birth rate was calculated by live births < 37 weeks' gestational age per live births. The low birth weight rate was calculated by live births < 2,500 g per live births.

Statistical analysis

Mean age of patients was examined by using analysis of variance followed by the bonferroni procedure for multiple comparisons. Percentage data was analyzed by using X^2 test or the Fischer exact test on a case-by-case basis. Selection bias exists when the number of embryos to transfer is studied in a retrospective fashion.

Results

The background of the patients is shown in (Table 1). There were no significant differences in respect to cycle number, mean age, duration of infertility, FSH and LH. Patients were assigned to one of three groups according to the scores of their vitrified-warmed blastocysts on thawing day. Patients in group 1 had two high-quality vitrified-warmed blastocysts for transfer (≥3BB). Patients in group 2 had one high-quality vitrified-warmed blastocyst (≥3BB) and one general-quality vitrified-warmed blastocyst (<3BB) for transfer, and patients in group 3 only had two general-quality vitrified-warmed blastocysts for transfer (<3BB).

The vitrified-warmed blastocyst score had a significant effect on implantation rate ((Table 2), $P < 0.05$). Fetal heart pregnancy rate showed a positive correlation to the quality of vitrified-warmed blastocyst. Live- birth rate of group 1 was significantly higher than group 3 ($P < 0.01$). Although there were no significant differences in multiple birth rate among three groups, transfer of two high-quality vitrified-warmed blastocysts resulted in multiple birth rate up to 44.4%.

In this analysis, neonatal outcomes of seven patients lost to follow up. There were no significant differences in the average live-birth weight among three groups, no matter for singleton or twins (Table 3). But it is noteworthy that the average live-birth weight of twins was significantly lower than that of singleton in each group ($P < 0.05$).

Discussion

Every zygote is endowed with a different inherent capacity to progress to further stages of embryonic development. The embryo that has the potential to develop into blastocyst will be easier to implant successfully [8]. So, blastocyst quality is one of the important factors that affect clinical outcomes, while the proportion of high quality blastocyst reduces markedly as patient grows older. In the study of Gardner, et al. [15], blastocyst scored more than "3AA" is the best selection for a single blastocyst transfer, but not all patients can obtain such a high quality blastocyst. Yanaihara, et al. [16] reported that both the clinical pregnancy rate and live-birth rate were not significantly different between single transfer and dual transfer in frozen-thawed embryo transfer cycle, in their research, the vitrified-warmed blastocyst were graded more than "3BB". In our study, assuming that the quality selection criteria of a single vitrified-warmed blastocyst transfer was 3BB, and higher fetal heart pregnancy rate and live-birth rate were attained. So our hypothesis was supported by the results of Yanaihara, et al.

From this analysis, it is obvious that the quality of human vitrified-warmed blastocyst assessed by a three-part scoring system can be used to identify viable embryos for transfer. There appears to be a strong correlation between the success of transfer and vitrified-warmed blastocyst quality. When a patient had two high-quality vitrified-warmed blastocysts available for transfer (group 1, ≥3BB), implantation rate and fetal heart pregnancy rate of 48.2% and 65.5% were attained, with a 44.4% incidence of twins. Therefore, it is recommended that a single blastocyst transfer in patients with at least one high-quality vitrified-warmed blastocyst (≥3BB).

Studies indicated that controlled ovarian stimulation affects the luteal phase function and alerts the endometrial development [17,18]. The comparison of controlled ovarian stimulation and natural cycles revealed the changes of endometrial gene and protein expression signatures which led to abnormal endometrial receptivity [19,20]. Therefore, identifying the most appropriate window of endometrial preparation and receptivity for embryo transfer is considered to be a crucial step. Endometrial receptivity may be improved by using the freeze-all policy [21]. Cyropreservation has become an indispensable ART procedure for both the effective use of surplus embryos and the prevention of ovarian hyper stimulation syndrome. Since embryo selection is made at the blastocyst stage rather than the cleavage stage, this factor may improve the clinical success rate; therefore, higher quality blastocyst should be chosen for transfer. Vitrified-warmed blastocyst transfer is useful under these circumstances. Multiple pregnancies certainly increase the risk of prematurity, prenatal morbidity and mortality. The current trend for ART is to reduce the risk of multiple pregnancies; thus, the utilization of single vitrified-warmed blastocyst transfer will increase.

Conclusion

This analysis indicated that a single vitrified-warmed blastocyst (scored more than 3BB) transfer can help to minimize the occurrence

of twin gestations, avoid the complications associated with such pregnancies and maintain acceptable overall live-birth rate. Moreover, the results will be benefit to a prospective and randomized controlled trial of a single vitrified-warmed blastocyst transfer.

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