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Original Article

Clinical outcomes comparison of single fresh and frozen-thawed superior blastocyst transfer

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Abstract: Objectives: To compare the clinical outcomes of single fresh and frozen-thawed superior blastocyst transfer in patients for the first transfer. Methods: This study included 770 single fresh blastocyst and 639 single frozen-thawed blastocyst transfers from January 2013 to January 2016. All the fresh and frozen-thawed embryo transfer was first transfer for the patients. The patients were divided into fresh group and frozen-thawed group. Main outcome measure: female age, endometrial thickness, infertility duration, clinical pregnancy, ectopic pregnancy and abortion rate. Results: We observed that there was no significant difference in female age, endometrial thickness, and infertility duration and abortion rate between two groups. It's worth noting that the clinical pregnancy rate in fresh group was lower than frozen-thawed group (66.36% vs 68.39%, P = 0.420) and the ectopic pregnancy rate was higher in fresh group than frozen-thawed group (1.57% vs 0.46%, P = 0.096). Conclusions: The data support the view that the frozen-thawed transfer may be a better clinical strategy for patients with superior blastocyst in their first transfers.

Keywords: Blastocyst, pregnancy, fresh transfer, frozen-thawed transfer

Introduction

In the last 3 decades the twin pregnancy rate within the overall ART population has increased by 50% to 70% which might be due that more ART centers have switched from D3 (D3) embryo transfer to day-5/6 (D5/6) blastocyst transfer [1, 2]. There is no doubt that the implantation and clinical pregnancy rate after blastocyst stage embryo transfer appeared superior to cleavage-stage embryo transfer [3, 4]. One main reason was that the activation of embryonic genome happens at the eight-cell stage [5]. An obvious way to ensure activation is to extend the duration of culture to blastocyst stage.

An extensive body of literature has reported that twin gestations carry a high risk of poor clinical outcomes, such as prematurity, infant mortality, and maternal mortality [6, 7]. As for the high implantation ability of blastocyst, elective single blastocyst transfer (eSBT) has increasingly been recommended to reduce the rate of multiple births. Especially for younger patients (aged < 36 years), elective single blastocyst transfer should be selected to reduce multiple pregnancy rates [8, 9].

To some extent, a single blastocyst transfer might decrease the pregnant probability compared with the transfer of 2 blastocysts. So for single balstocyst transfer, the embryo should have a high score according to the blastocyst grade criteria. A recent study showed that embryo quality is the main factor affecting cumulative live birth rate after elective single embryo transfer [10]. In our center, extended culture has been considered for the patients who have more superior D3 embryo. Generally speaking, such patients have a high number of retrieved oocytes and some patients should require whole embryo freezing for some reasons. Of course, the fresh transfer would be performed on day 5 for most patients. With this in mind, we aimed to compare the clinical outcomes of single fresh and frozen-thawed superior blastocyst transfer in patients for the first transfer.

Table 1. Blastocyst grade criteria

	Rating	Description
Development stage	1	Degree of expansion and hatching status
		An early blastocyst with a blastocoel that is less than half the volume of the embryo
	2	A blastocyst with a blastocoel that is half, or greater than half, of the volume of the embryo
	3	A full blastocyst with a blastocoel completely filling the embryo
	4	An expanded blastocyst with a blastocoel volume larger than that of the early embryo, with a thinning zonapellucida
	5	A hatching blastocyst with the trophectoderm starting to herniate though the zonapellucida
	6	A hatched blastocyst which has completely escaped from the zonapellucida
Inner cell mass	A	Tightly packed with many cells
	B	Loosely grouped with several cells
	C	Very few cells
Trophectoderm	A	Many cells forming a cohesive epithelium
	B	Few cells forming a loose epithelium
	C	Very few large cells

Materials and methods

This study was a retrospective analysis of the data from our center and was approved by the Ethics Review Board of Northwest Women's and Children's Hospital. This study included 770 fresh single blastocyst and 639 frozen-thawed single blastocyst transfers from January 2013 to January 2016. Women patients were recruited in accordance with the following criteria: aged < 40 y, using non-donor oocytes and in receipt of their first fresh or frozen-thawed transfer; ≥ 4 good-quality embryos on D3; the transfer involved blastocysts on D5; normal endometrium.

Ovarian stimulation protocol and frozen-thawed policy

Most patients received standard long and short protocols featuring a gonadotropin-releasing hormone (GnRH) agonist (GnRH-a, Decapeptyl, Germany) and recombinant follicle-stimulating hormone (FSH; GONAL-f, Merck Serono, Italy; Puregon, Organon, Netherlands) for controlled ovarian hyper-stimulation. Other protocols, with or without human menopausal gonadotrophin (Li Zhu, China), were also adopted for controlled ovarian hyper-stimulation according to the patient's response to stimulation, with 10 000 units of human chorionic gonadotrophin administered when > 3 follicles were > 18 mm in size. Oocyte retrieval was performed 36 h later by trans-vaginal ultrasonography-guided aspiration [11].

In this study, patients' surplus embryos were first vitrified on Day 5. The frozen policy was carried out in those patients who failed to conceive from fresh ET. In patients who were at a risk of ovarian hyperstimulation syndrome (OHSS), or presented with an endometrial cavity fluid complication, hydrosalpinx, abnormal endometrium or acute marital problems, noembryos were transferred and all were frozen on Day 5 and 6. The frozen embryos were warmed and transferred usually after a few monthsfollowing oocyte collection.

Embryo freezing and warming procedures

The freezing and warming procedure was done according to standard protocols, as previously described [12]. Tools and solutions required for vitrification were obtained from Kitazato (Kitazato Bio Pharma Co, Japan). The thawed embryos were observed after warming and againbefore transfer to assess for morphological survival.

D5 embryo grading

On D5, the embryos were scored using published criteria [13] (**Table 1**). A single embryo was transferred when more than one high-quality embryo (4 ≥ BB) was available. The embryos graded 4BB, 4BA, 4AB and 4AA were concluded in this study. After embryo transfer, all patients were provided with luteal support (Duphaston; progesterone injection). Clinical pregnancy was confirmed by the existence of a gestational sac.

Statistical analysis

Data were analyzed using the SPSS 17.0 for Windows (SPSS Inc., Chicago, IL). When comparing means between different groups, the

Table 2. Comparison of basic characters and clinical outcomes in fresh and frozen-thawed group

Parameter	Fresh group	Frozen-thawed group	*P* value
Single blastocyst cycle (total, n)	770	639	/
Female age (yr)	29.14 ± 3.37	29.43 ± 3.56	0.389
Endometrial thickness (mm)	11.22 ± 2.09	11.35 ± 2.21	0.476
Infertility duration (yr)	3.68 ± 2.59	3.58 ± 2.47	0.401
Clinical pregnancy (%, n)	66.36 (511/770)	68.39 (437/639)	0.420
Abortion (%, n)	8.41 (43/511)	8.92 (39/437)	0.781
Ectopic pregnancy (%, n)	1.57 (8/511)	0.46 (2/437)	0.096

paired Student's t-test was applied. The χ^2 test was used for group comparison of rate. Differences were considered statistically significant at $P < 0.05$.

Results

For the entire study, 770 fresh single blastocyst and 639 frozen-thawed single blastocyst transfers were concluded. The distribution of cycle-specific parameters between groups is described in **Table 2** and no significant differences were observed in female's age, endometrial thickness and infertility duration ($P > 0.05$). The clinical pregnancy rate in fresh group was lower compared with frozen-thawed group (66.36% vs 68.39%, $P = 0.420$) and the ectopic pregnancy rate was higher in fresh group than frozen-thawed group (1.57% vs 0.46%, $P = 0.096$). The abortion rate was no significant difference between fresh group and frozen-thawed group (8.41% vs 8.29%, $P = 0.781$).

Discussion

It is well known that safety is the common concern of ART technology and it has been a research hotspot in how to reduce the incidence of multiple pregnancy [14]. Improved laboratory standards and better culture media have made extended culture to blastocyst stage a reality to identify embryos with maximum implantation potential. So the strategy of extended culture has become more popular around the world at a time when regulatory bodies have emphasized the need to increase the uptake of elective single embryo transfer, minimize complications associated with multiple births [15].

Some studies' results showed that the clinical pregnancy rate might not be affected if the quality of blastocyst was superior and the multiple pregnancy rate would be reduced for elective single embryo transfer [16-18]. So for single embryo transfer, the selection for transferrable embryo is significant. Generally speaking, the grade of inner cell mass and trophectoderm is valuable for the embryologist's option. In our center, if the patients is not more than 36 years old and has more than one blastocyst (4 ≥ BB), we usually perform elective single embryo transfer in their first cycles. Of note, our primary aim in the present study was to compare the clinical outcomes of single fresh and frozen-thawed superior blastocyst transfer in patients for the first transfer.

In this study, we observed that the clinical pregnancy rate in fresh group was lower compared with frozen-thawed group and the ectopic pregnancy rate was higher in fresh group than frozen-thawed group which suggested that the frozen-thawed transfer might be more suitable for patients with superior blastocyst in their first transfers. It also reminds us that the physical condition of few patients might not be in good state for fresh transfer, though the ovarian hyper-stimulation syndrome was not found in the transfer day. And a large number of studies have confirmed that the incidence of ectopic pregnancy rate might be decreased in frozen-thawed transfers than in fresh transfers [19, 20].

Blastocyst transfer has become the strategy of choice for most reproduce centers worldwide, with the aim of achieving a healthy singleton live-birth and so minimizing the number of multiple births. However, it is still equally important to maintain clinical pregnancy rates per transfer. Summarizing our results, we concluded that the frozen-thawed superior blastocyst transfers have some advantage than fresh transfers in the aspects of clinical and ectopic pregnancy rate. Nevertheless we there was no significant difference between two groups because of the limited cases. So in the future work, it is more important to accumulate clinic data for a more persuasive conclusion.

Disclosure of conflict of interest

None.

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