Clinical outcome of frozen blastocyst transfer; single vs. double transfer

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Abstract

Purpose Frozen embryo transfer has been established as an indispensable ART procedure for both the effective use of surplus embryos and the prevention of ovarian hyperstimulation syndrome. The frequency of frozen embryo transfer is increasing in our clinic, and we report that frozen embryo transfer is effective for patients with repeat failures. We present our clinical outcome of frozen blastocyst transfer (FBT).

Methods In 2006, 470 patients received FBT (562 cycles (IVF: 354 cycles; ICSI: 208 cycles)). One frozen blastocyst was transferred in 412 cycles (335 patients) and two blastocysts were transferred in 150 cycles (135 patients). Assisted hatching was performed in all cases.

Results In 412 cycles (average age: 34.6 years) who received a single FBT, the rate of clinical pregnancy per cycle was 40.7%, the live birth rate was 29.1%, the abortion rate was 21.6%, the ectopic pregnancy rate was 1.2%, the frequency of monochorionic twins was 2.3%, and the cesarean section rate was 38.3%. In 150 cycles (average patient age 34.8 years) who received two FBTs, the clinical pregnancy rate was 46%, the live birth rate was 35.3%, the abortion rate was 16.3%, the ectopic pregnancy rate was 4.4%, the frequency of twins was 15.9% and the cesarean section rate was 39.6%. A significant difference in the ectopic pregnancy rate and the twinning rate was found between single transfers and double transfers ($P < 0.05$). When IVF and ICSI were compared, there was no

statistically significant difference in the abortion rate, the ectopic pregnancy rate, and the cesarean section rate.

Conclusions The clinical pregnancy rate was similar for the transfer of one and two blastocysts. Single FBT decreases obstetrical risk without reducing the pregnancy rate.

Keywords Blastocyst · Vitrification · In vitro fertilization · Pregnancy

Introduction

Cryopreservation has become an important assisted reproductive technology (ART) procedure. Frozen-thawed embryo transfer appears to not only reduce the risk of multiple gestation but also avoid the ovarian hyperstimulation syndrome [1, 2]. Blastocyst culture techniques have improved and the blastocysts can be cryopreserved by vitrification [3]. A number of investigators have reported superior clinical results with blastocyst transfer (BT) rather than transfers using cleavage-stage embryos [4–6]. Moreover, it has been reported that BT may be efficacious in cases of repeated pregnancy failure with cleavage stage embryo transfer [5, 6]. In women over the age of 35, single ET has also produced satisfactory results [7]. 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 embryos should be chosen for transfer. Frozen-thawed blastocyst transfer (FBT) is useful under these circumstances. As the rate of twinning is high with dual transfer, this certainly increases the risk of prematurity and therefore the prenatal morbidity and mortality. The current trend for ART is to reduce the risk of multiple pregnancies; thus, the utilization of single FBT will increase. In this study, a comparison has been made

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Table 1 Background of patients

	Single	Double
Duration of infertility (m)	36.7	43.4
No. of Blastocysts available for fresh transfer	0.72	0.78
No. of frozen blastocysts	3.3	4.2
ET (Natural vs HRT)	79% vs 21%	71% vs 29%

between single FBT and double FBT with respect to pregnancy outcome.

Material and methods

In 2006, a total of 562 cycles (IVF: 354 cycles; ICSI: 208 cycles) were conducted on 470 patients using frozen blastocyst transfer. Among 335 patients with 412 cycles (average age: 34.6 ± 3.4 years), single FBT cycles were conducted and among 135 patients with 150 cycles (average age 34.8 ± 3.7 years), double FBT cycles were conducted with 92 IVF cycles and 58 ICSI cycles. The indications for IVF before frozen embryo transfer were tubal infertility, mild male factor infertility, unexplained infertility, early stage endometriosis, and the polycystic ovary syndrome. Background of each group of patients is shown in Table 1. The couples opted for either the mild stimulation protocol or the long protocol and full informed consent was obtained. In order to preserve surplus embryos and to avoid the ovarian hyperstimulation syndrome, blastocysts were frozen with vitrification using Cryotop (KITAZATO Co. BioPharma, Shizuoka, Japan) according to the manufacturer's specifications [3]. The embryos that were graded more than "3BB" according to the Gardner's classification were frozen.

Frozen blastocysts were warmed to 37°C in VT102 (KITAZATO Co. BioPharma, Shizuoka, Japan) and cultured with BlastAssist® (MediCult, Jyllinge, Denmark) for 3–4 h before transfer. Assisted hatching was performed on almost all cases via modified long zona dissection (LZD) [8]. Gardner's classification was used for embryo scoring before transfer [9]. A single blastocyst was transferred on day 5 of a natural cycle or day 6 of a hormone replacement cycle [10, 11]. For endometrial preparation, Estrana® (Hisamitsu Pharmaceutical Co. Inc., Saga, Japan) was begun on the third menstrual day and continued until endometrial thickness was >8 mm. There was no difference between endometrial thicknesses between the two groups.

Luteal support was begun 6 days before transfer and continued for 2 weeks (a 222 mg progesterone vaginal suppository was inserted every 12 h and 125 mg of hydroxyprogesterone caproate was injected intramuscularly once a week). A clinical pregnancy was defined as a

gestational sac on ultrasound. The chi square test and Student's *t* test were used for statistical analysis, and $P < 0.05$ was defined as statistically significant.

Result

The background of the patients is shown in Table 1. Although there was statistical significance in duration of infertility, there was no significance in the number of blastocysts available for fresh transfer, the number of frozen blastocysts in each and the number of natural or HRT cycles.

The results for total of 502 single FBT and dual FBTs are presented in Table 2. In the single FBT, the clinical pregnancy rate per cycle was 168/412 (40.7%), the abortion rate is 47/218 (21.6%), the ectopic pregnancy was 2/168 (1.2%), the frequency of monozygotic twins was 4/168 (2.3%), and the cesarean section rate was 46/168 (27.3%).

For the two FBT transfers (150 cycles; average patient age 35.8 ± 3.7 years), the clinical pregnancy rate was 69/150 (46%), the abortion rate was 13/80 (16.3%), the ectopic pregnancy rate was 3/69 (4.4%), the frequency of twins was 11/69 (15.9%), and the cesarean section rate was 21/69 (30.4%). The frequency of monozygotic twins was 0%. There was a significant difference in both the ectopic pregnancy rate and the twinning rate between single embryo transfer and dual embryo transfer ($P < 0.05$). When IVF and ICSI were compared, there was no statistically significant difference in the abortion rate, the ectopic pregnancy rate, and the cesarean section rate (Table 2).

Discussion

In this study, comparison of clinical outcome of FBT between single transfer and dual transfer was performed. Both the clinical pregnancy rate and live birth rate were

Table 2 Clinical outcome of FBT; single vs double

	Single	Double
Cycle	412	150
Age	34.6 ± 3.4	34.8 ± 3.7
Previous IVF	2.6	2.9
ICSI vs IVF	150 vs 262	58 vs 92
Clinical preg(%)	40.7	46
Live birth rate(%)	29.1	35.3
Abortion rate(%)	21.6	16.3
Ectopic preg(%)	1.2*	4.4*
Twinning rate(%)	2.3*	15.9*
Cesarean section(%)	38.3	39.6

* $p < 0.05$

higher in dual transfer, however, the difference was not statistically significant. When the ectopic pregnancy rate and twin pregnancy rate were compared, they were significantly higher in dual transfer group than in single transfer group.

It is thought that the embryo physiologically reaches the endometrium 5 days after ovulation; thus, blastocyst transfer on day 5 is logical. The stage at which embryo transfer is conducted can be selected based upon the patient's needs and clinical circumstances. The literature contains a number of papers that compare the pregnancy rate of transfers at the blastocyst stage to that of the cleavage stage; these papers also address the efficacy of blastocyst transfer [4–6]. Blastocyst transfer has a higher implantation rate than that of cleavage stage transfer and is effective for cases with repeated pregnancy failures [5, 6].

Recently, a French group reported no difference in pregnancy rate between single embryo transfer and double embryo transfer [13]. They did not report any monozygotic (MZ) twins following 107 single embryo transfers; however, we noted a 1.55% incidence of MD twins. This rate is higher than that of spontaneous gestation. Although the discrepancy in regard to MD twins between our group and the French group is unclear, blastocyst transfer may raise the rate of MD twins [14]. Furthermore, the literature contains a few reports addressing the cause of MD twins [15, 16]. Because of small numbers, there may be no monozygotic gestations in a double transfer.

With recent improvements in culture media, blastocyst transfer has become established as an embryo transfer technique; furthermore, frozen embryo transfer has also evolved. Although the slow freezing method is used for cleavage stage embryos, the vitrification method can readily be employed at the blastocyst stage [3].

Freezing the embryo results in hardening of the zona pellucida; however, this problem can be resolved by assisted hatching (AH). It has been reported that the pregnancy rate of a frozen-thawed embryo increases with AH [12]. In our clinic, the modified partial zona dissection (PZD) method is used on the blastocysts for AH [7].

Previously, two or more embryos were transferred in order to increase the pregnancy rate; however, the current trend is to transfer fewer embryos to minimize obstetrical risk, such as multiple gestations, while maintaining a high pregnancy rate.

A number of investigators have reported that the pregnancy rate increased with a multiple transfer [2, 17]. If there is no significant difference in the pregnancy rate and the twinning rate increases with a double transfer, single transfer is the preferred method. Furthermore, the incidence of ectopic pregnancies increases with a double transfer. It has been suggested that tubal infertility is a major factor for the utilization of in vitro fertilization; thus,

increasing the number of embryos transferred increases the risk of an ectopic pregnancy. With the use of a good-quality blastocyst following freezing, single FBT can achieve a comparable pregnancy rate to that of a double FBT. Moreover, single FBT can reduce the incidence of twins and ectopic pregnancies.

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