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At what stage does the embryo begin to grow larger in frozen-thawed embryo transfers?: fetal development from the standpoint of gestational sac diameter and birth weight

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Abstract: Frozen-thawed embryo transfers, whose number has risen considerably in recent years, reportedly result in heavier birth weights than fresh embryo transfers. To find out what this difference means and the stage at which it becomes manifest during fetal development, we studied birth weight and gestational sac size, which reflects development immediately following implantation, in 365 single pregnancies employing fresh embryo transfer and 227 employing frozen-thawed embryo transfer. Comparison of fresh embryo transfers and frozen-thawed embryo transfers revealed that average birth weights were significantly higher in the latter, with average values ± SD of 2896.0 ± 515.7 g and 3060.0 ± 529.2 g, respectively. Transvaginal ultrasound showed significantly larger average gestational sac diameters at 21, 22, 23, 28, 29 and 30 days after fertilization in frozen-thawed embryo transfers. We speculate these results are explained mainly by hormone replacement therapy in frozen-thawed embryo transfer cycles exerting a more positive influence on the endometrium, promoting smoother implantation, greater development during early pregnancy, and significant increases in birth weight. Amidst concerns regarding the impact exerted on fetuses by the artificial operations entailed by in vitro fertilization and embryo transfers, these findings may serve as evidence of the safety of frozen-thawed embryo transfers.
Key words: frozen-thawed embryo transfer, Fresh embryo transfer, Birth weight, Gestational sac

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Introduction

According to calculations by the Japan Society of Obstetrics and Gynecology, the number of frozen-thawed embryo transfer cycles in Japan has exceeded 110,000, giving rise to the birth of over twice the number of babies conceived in fresh embryo transfers [1]. Advantages that can be expected from frozen-thawed embryo transfer include prevention of multiple pregnancies, avoidance of ovarian hyperstimulation syndrome, and increased cumulative pregnancy rates, making it an essential technology in reproductive medicine. In recent years, it has become clear that frozen-thawed embryo transfers lead to increased birth weight compared to fresh embryo transfers [2–10]. Discovering the causes behind this increase and the stage at which the difference becomes manifest during fetal development may be helpful in determining whether frozen-thawed embryo transfers are safe. We therefore compared frozen-thawed embryo transfers and fresh embryo transfers in order to determine differences in gestational sac size, and to investigate birth weight, birth height and development immediately after implantation.

Materials and Methods

Upon obtaining informed consent from patients receiving ART at our clinic, over the period from April 1998 to May 2010, we compared birth weight, birth height and low birth weight rates in 365 single pregnancies employing fresh embryo transfer (Fresh-ET) and 227 single pregnancies employing frozen-thawed embryo transfer (FET). We also compared birth weights in subgroups sorted by gender, whether IVF or ICSI was employed,

and whether an early cleavage stage embryo transfer or a blastocyst transfer was performed. Furthermore, we employed bidirectional averages of gestational sac diameter in Fresh-ET and FET in order to detail the period immediately following implantation. One experienced doctor, the first author, performed all ultrasound scans using the Prosund α7 (Hitachi Aloka Medical Ltd., Tokyo, Japan) with a 5-MHz transvaginal transducer.

During egg collection cycles, we began ovarian stimulation on the 3rd day of the menstrual period by employing pure FSH (Fertinorm®P; Merck Serono, Geneva, Switzerland) and hMG (Humegon® ; Organon, Holland), and administered hCG (Profasi®; 5000, Merck Serono, Geneva, Switzerland) once the diameter of the dominant follicles exceeded 18 mm. GnRH antagonist Cetrorelix (Cetrotide® 0.25 mg; Merck Serono, Geneva, Switzerland) was used to suppress premature LH secretion in some cases. Eggs were collected 36 h later, IVF or ICSI was performed, and their incubation was continued after confirming fertilization. For Fresh ET, we employed early cleavage stage embryos or blastocysts after 3 or 5 days of incubation, respectively. On the 3rd day of egg collection, we began intravaginal administration of micronized progesterone (Utrogestan®; Besins Manufacturing Belgium, Drogenbos, Belgium), 200 mg twice/daily, as a form of luteal support, and continued this treatment until the 9th week of pregnancy.

Early cleavage stage embryos and blastocysts were cryopreserved by slow freezing or vitrification. We employed slow freezing in 119 cases, and vitrification for the rest. The slow freezing was performed with controlled rate freezing using 1.5 M propanediol and 0.1 M sucrose solutions as cryoprotectants, and the embryos were stored in liquid nitrogen. After slow cooling at 2 °C/min the embryos were seeded at −7 °C, cooled at 0.3 °C/min to −30 °C, and at 5 °C/min to −100 °C before final transfer to liquid nitrogen for storage. The program freezer used was Cryoembryo HP (Daido hoxan, Sappro, Japan). The vitrification was carried out using ethylene glycol (7.5%, 15%) and dimethyl sulfoxide (7.5%, 15%) as cryoprotectants. After rapid treatment with cryoprotectants, the embryos were placed on the carrier Cryotop (Kitazato, Tokyo, Japan), vitrified with ultra rapid cooling, and stored in liquid nitrogen.

For the endometrial adjustments made when transferring thawed cryopreserved embryos, we administered nose drops of a GnRH agonist (Nafarelil®; Fuji Pharma Co. Ltd., Tokyo, Japan) twice every day from 1 to 2 weeks before the beginning of menstruation for the transfer's scheduled cycle until the 6th day of the menstrual period. An estradiol patch (Estrana®; Hisamitsu Pharmaceutical Co., Inc. Tokyo, Japan) was attached every other day from the 3rd day of the menstrual period onwards and 600 mg of micronized progesterone (Utrogestan®) were also administered every day intravaginally from the 17th day of the menstrual period onwards. Also, 125 mg of hydroxyprogesterone capronate (Oophormin luteum depot®; ASKA Pharmaceutical. Co., Ltd., Tokyo, Japan) were injected intramuscularly on the 17th, 19th and 24th day of the menstrual period. Cryopreserved early cleavage embryos were thawed on the 20th day of the menstrual period and transferred on the same day. Cryopreserved blastocysts were thawed on the 22nd day of the menstrual period and transferred on the same day.

The significance of differences between groups were investigated using Student's t-test.

Results

Gestational ages of 38.2 ± 2.0 vs 38.2 ± 2.4 weeks showed no significant difference between the Fresh-ET group and the FET group, and no significant differences were found in subgroups of gestational ages, either (Table 1). Mothers' ages of 33.3 ± 4.1 vs 33.4 ± 3.9 years showed no significant difference between the groups, and no significant differences were found in subgroups of ages, either (Table 2).

The Fresh-ET group and the FET group had average birth weights (±SD) of 2896.0 ± 515.7 vs 3060.0 ± 529.2 g ($P < 0.0005$) and average birth heights (±SD) of 47.6 ± 2.0 vs 48.5 ± 1.5 cm ($P < 0.005$). Babies in the FET group were significantly larger than babies in the Fresh-ET group. LBW (low birth weight:<2500 g) and VLBW (very low birth weight:<1500 g) rates were 17.3% vs 11.3% ($P < 0.05$), and 2.1% vs 1.8% ($P < 0.05$), respectively - significantly lower in the FET group than in the Fresh-ET group. The FET group had a slightly higher rate of babies weighing over 4000 g (2.7% vs 4.4%). Comparison of the slow freezing and vitrification methods within the FET group revealed there was no significant difference in the average birth weights (±SD) of 3092.0 ± 498.6 and 3031.6 ± 565.9 g, respectively (Fig. 1).

Birth weights of the Fresh-ET and FET groups were compared by gender, and the values of the FET group were significantly higher for both male (2898.4 ± 544.2 vs 3120.5 ± 530.7 g) and female babies (2885.8 ± 464.3 vs 2993.3 ± 519.4 g) (Fig. 2).

Birth weight comparisons of IVF and ICSI, showed they were significantly higher for both methods in the FET group: IVF, 2907.7 ± 488.7 vs 3081.9 ± 534.6 g, and ICSI, 2883.8 ± 519.4 vs 3041 ± 479.0 g, respectively (Fig. 3).

Table 1. Pregnancy duration in Fresh-ET and FET

Pregnancy duration (in weeks)	Number of cases (%)	
	Fresh-ET	FET
≥42	0 (0.0)	3 (1.3)
37~41	323 (88.5)	190 (83.7)
34~36	33 (9.0)	27 (11.9)
≤33	9 (2.5)	7 (3.1)

No significant differences were found between the groups for any of the gestational age ranges.

Table 2. Age of mothers in Fresh-ET and FET

Age (years)	Number of cases (%)	
	Fresh-ET	FET
≥40	22 (6.0)	13 (5.7)
35~39	127 (34.8)	76 (33.5)
30~34	143 (39.2)	101 (44.5)
25~29	70 (19.2)	35 (15.4)
≤24	3 (0.8)	2 (0.9)

No significant differences were found between the groups for any of the age ranges.

Early cleavage stage embryo transfers and blastocyst transfers, likewise resulted in significantly higher birth weights in the FET group: early cleavage stage embryos, 2889.6 ± 511.9 vs 3050.3 ± 533.5 g, and blastocysts, 2917.5 ± 527.6 vs 3144.4 ± 412.9 g, respectively (Fig. 4).

Finally, as shown in Fig. 5, the gestational sac at the beginning of pregnancy had a significantly larger diameter in the FET group than in the Fresh-ET group at 21, 22, 23 and 28, 29 and 30 days after fertilization (Fig. 5).

Discussion

Since the beginning of in vitro fertilization and embryo transfers, most transfers performed have been Fresh-ET carried out during an ovarian stimulation cycle. Success rates, however, are still unsatisfactory, and improvement is required. The possible causes of failure are many. Recently, however, it is becoming clear that the uterine environment, including the endometrium, does not lend itself well to implantation and early development during stimulation cycles [11]. The considerable progress of embryo freezing techniques in recent years has enabled the achievement of extremely high resuscitation rates when thawing cryopreserved embryos. The number of FET, which avoid ovarian stimulation cycles and instead en-

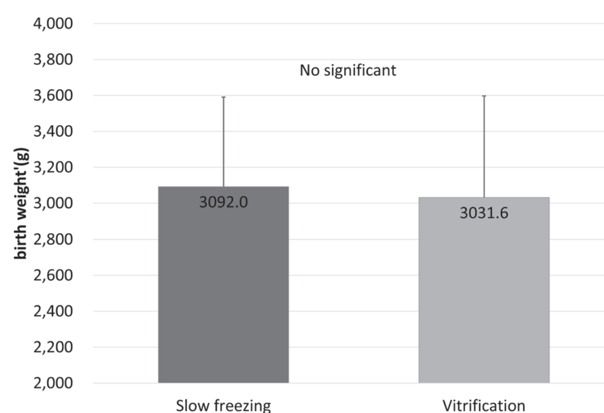

Fig. 1. Comparison of birth weights of different freezing techniques. No significant difference in birth weight was found between slow freezing and vitrification.

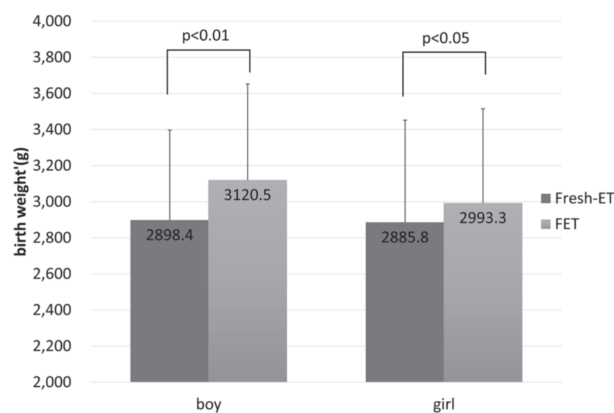

Fig. 2. Comparison of birth weight by gender. Birth weight was significantly heavier for both genders in FET than in Fresh-ET.

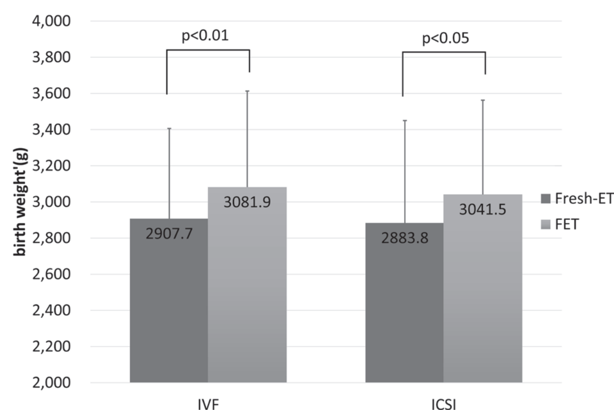

Fig. 3. Comparison of birth weights of different fertilization methods. Birth weight was significantly heavier in FET than in Fresh-ET in both IVF and ICSI.

tail transferring the embryo to the uterus during natural cycles or hormone replacement cycles, is therefore rising rapidly. FET, in which the embryo is transferred to the uterus in a more favorable environment than in ovarian stimulation cycles, can be expected to dramatically boost hitherto stagnant success rates. In actual fact, the clinical pregnancy rates and the live birth rates per transfer of Fresh-ET and FET are 20.8% vs 33.7% and 14.0% vs 22.9%, respectively, and the success rates of FET exceed those of Fresh-ET according to a report by the Japan Society of Obstetrics and Gynecology [1]. Further advantages of FET are the possibility of avoiding ovarian hyperstimulation syndrome, and of preventing multiple pregnancies with single embryo transfers. The collection of multiple good quality embryos also reduces the burden placed on the patient as it enables multiple transfers from a single egg collection and incubation.

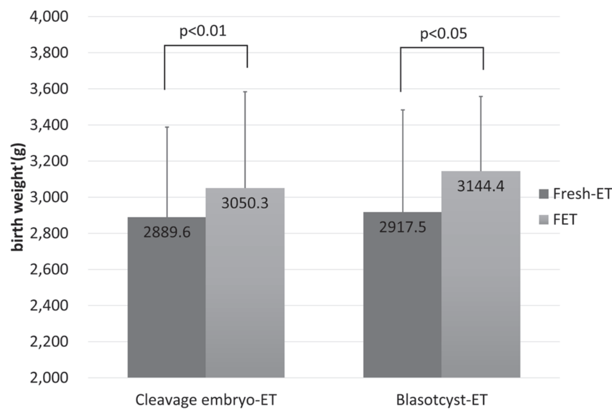

Fig. 4. Comparison of birth weights of early cleavage stage embryo transfers and blastocyst transfers. Birth weight was significantly heavier in FET than in Fresh-ET in both early cleavage stage embryo transfers and blastocyst transfers.

Although FET singletons have perinatal outcomes comparable with those observed in naturally conceived singletons [3, 6, 12] and no cases of FET affecting seriously either the mother`s body or the embryo have been reported, its safety has yet to be established. In this study, we compared the Fresh-ET that has traditionally been performed with more recent FET in terms of single embryo development. We found that the birth weight was significantly heavier in FET, and that the gestational sac was significantly larger in frozen-thawed embryo pregnancies from the very early stage of 21 days after fertilization. Our result that birth weight was significantly heavier in FET than in Fresh-ET, is consistent with previous reports [2–10]. Reports that closely measure early-stage gestational sac size for each pregnancy, however, are few, and, to our knowledge, this study was the first to measure and compare the diameter of the gestational sac during this stage in Fresh-ET and FET. The fact that

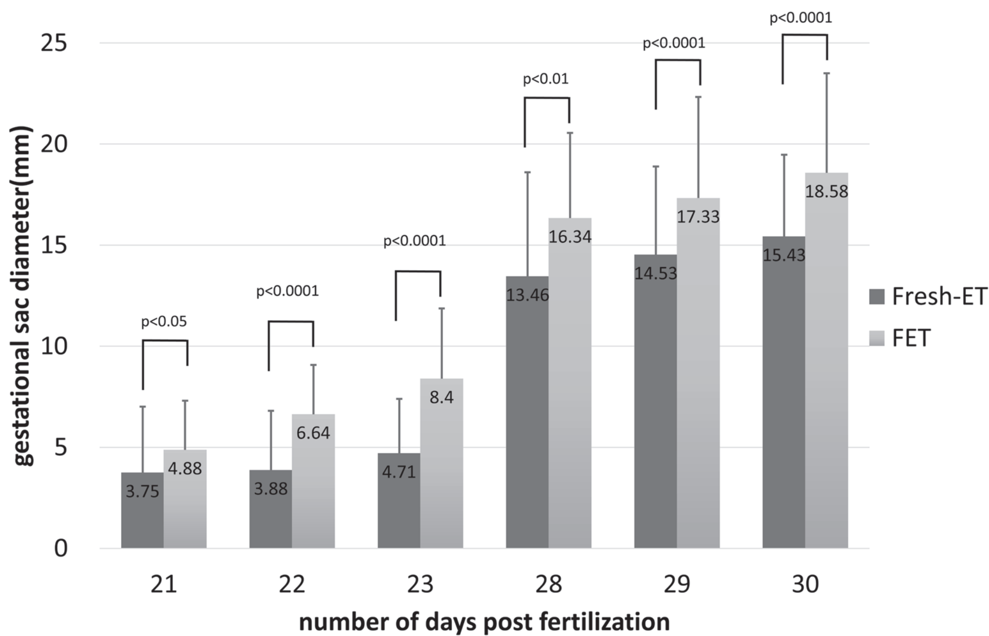

Fig. 5. Comparison of gestational sac diameters of Fresh-ET and FET. Gestational sac diameter was significantly heavier in FET than in Fresh-ET from the very early stage of 21 days after fertilization.

the gestational sac diameter was significantly larger in the earliest stages of pregnancy compared to Fresh-ET, and that birth weight was heavier, suggests that the uterus offers a more favorable environment for implantation and early embryo development than ovarian stimulation cycles, and that implantation and gestational sac formation occur more smoothly than in Fresh-ET. Furthemore, the data presented here probably imply that the cryopreservation procedures enable the embryo to regain full viability and attain smooth early stage development and might change the developmental potential of the embryo. These findings support the view that FET is safe.

FET is a new treatment that involves a number of interventions and there is a source of concern regarding the impact exerted on the embryo. The results of the present study may serve as evidence of the safety of FET.

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