Effect of embryo and blastocyst transfer on the birthweight of live-born singletons from FET cycles

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Received: 14 April 2018 / Accepted: 2 July 2018 / Published online: 20 July 2018
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Abstract

Purpose To evaluate the effect of culture duration (embryo (day 3) transfer vs. blastocyst (day 5–6) transfer) on the birthweight of singletons from frozen embryo transfer (FET) cycles.

Methods A total of 1092 singletons were analyzed in this retrospective study. The distribution of large for gestational age (LGA) infants, the mean birthweight, and *z* scores of singletons were compared between the day 3 and day 5–6 transfer groups. Multiple linear regression analysis was performed to evaluate the relationships between confounding factors and singleton birthweight.

Results The proportion of LGA infants significantly increased with BMI (BMI < 20, 12.8%; 20 ≤ BMI ≤ 25, 23.2%; BMI > 25, 32.3%; *P* < 0.0001). However, the proportions of small for gestational age (SGA) and LGA infants were not significantly different between day 3 and day 5–6 transfers. The absolute mean birthweight of singletons was not significantly different between day 3 transfer (3422 ± 547 g) and day 5–6 transfer (3433 ± 559 g; *P* = 0.732). The *z* scores (calculated from a reference population) of singletons were also not significantly different between the two groups (0.499 vs. 0.533, *P* = 0.625). Multiple linear regression analysis showed that maternal BMI, gestational age, and infant gender had significant effects on singleton birthweight, while culture duration (*P* = 0.731) did not significantly affect singleton birthweight.

Conclusions In vitro culture duration did not affect the birthweight of newborns resulting from day 3 to day 5–6 transfers in FET cycles.

Keywords Embryo · Blastocyst · Birthweight · Singletons · FET

Introduction

Since the birth of the world's first in vitro fertilization (IVF) baby in 1978, approximately five million babies have been born through assisted reproductive technology (ART) [1]. However, increased risks of preterm birth and low birthweight have been reported in ART newborns compared with

newborns from spontaneous conceptions [2–4]. Several factors, including ovarian stimulation, the insemination method (IVF/ICSI), culture media composition and culture duration, freezing/thawing procedures, and number of embryos transferred, may impact perinatal outcomes. Multiple births due to transfer of more than one embryo can increase the risks of preterm birth and low birthweight. Meanwhile, single

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blastocyst transfer can significantly reduce the multiple birth rate and does not compromise the clinical pregnancy rate [5, 6].

Extended embryo culture has also been associated with increased risks of preterm birth and low birthweight [7, 8]. However, Mäkinen et al. reported an increase in large for gestational age (LGA) infants resulting from blastocyst transfer [9]. In addition, Mäkinen et al. and Zhu et al. observed that the absolute mean birthweight of singletons from fresh cycles was significantly higher among infants resulting from blastocyst transfer than among those resulting from embryo transfer [9, 10]. Meanwhile, other studies have reported that extended embryo culture in a fresh cycle does not affect singleton birthweight [11–14]. Therefore, the effect of in vitro culture duration in a fresh cycle on singleton birthweight is controversial. However, few studies have reported the effect of culture duration in a frozen embryo transfer (FET) cycle on singleton birthweight. The purpose of our retrospective study was to analyze the effect of culture duration on the birthweight of singletons by comparing infants resulting from day 3 with those resulting from day 5 to 6 transfer in FET cycles.

Materials and methods

Patients

All patients provided signed informed consent for FET treatment from January 2015 to June 2016 before participating in this study. One or two cleavage-stage embryos or blastocysts were transferred into the uterus no less than 2 h after warming on the day of FET. The inclusion criteria were patients with singleton pregnancies and singleton deliveries with gestational age no less than 28 weeks. Our retrospective study included 554 newborns from Day 3 transfer and 538 newborns from day 5 to 6 transfer (Fig. 1). The infants from vanishing twins, warmed oocytes, donor sperm, and non-ejaculated sperm were excluded from this study.

Definitions

All definitions, including singleton pregnancy, gestational age, preterm birth, low birth weight (LBW), very low birth weight (VLBW), small for gestational age (SGA) and LGA, were in accordance with the reported literature [9, 15–18].

FET and follow-up

Endometrial preparation for the FET was performed by a human chorionic gonadotropin (hCG)-induced natural cycle or a hormone replacement therapy (HRT) cycle. When the endometrium reached adequate thickness, this phase was complemented by administration of progesterone. The

vitrified-warmed embryos or blastocysts were transferred into the uterus on day 3–5 or day 6 of progesterone administration, respectively. Clinical pregnancy was confirmed by the presence of a gestational sac and fetal heartbeat by sonography at 42 days after FET. Data regarding neonatal birth date, gender, birthweight, and live birth or not were obtained during the follow-up.

Statistics

All data analyses were performed using SPSS 20.0 software. An independent samples *t* test was used to compare the means of the birthweight and gestational age of infants. The χ^2 -test was used to compare infant gender, prematurity rates, SGA rates and LGA rates. *P* values < 0.05 were considered statistically significant. *Z* score was used to evaluate the associations of birthweight and culture duration after adjustments for infant gender and gestational age. *Z* score = (newborn birthweight—the mean birthweight at the same gestational age for the same gender in the reference population)/the standard deviation (SD) in the same reference population. The reference population was derived from the latest publication regarding gestational age-specific singleton birthweight of Chinese infants [19]. Multiple linear regression analysis was used to evaluate the relationships between culture duration or other confounding factors (female age, BMI, pattern of infertility, duration of infertility, type of insemination in fresh cycles, stimulation protocol in FET cycles, thickness of endometrium, gestational age and infant gender) and singleton birthweight.

Results

The main maternal and treatment characteristics according to culture duration are shown in Table 1. No differences were observed between the Day 3 and Day 5–6 transfer groups except for female age and insemination method in a fresh cycle, which may be related to the higher blastocyst formation rate observed in younger women undergoing IVF.

Table 2 shows the perinatal outcomes of singletons. The mean birthweight ($\pm$ SD) of infants resulting from embryos transferred on day 5–6 was 3433 g ($\pm$ 559), which was not significantly different compared to the mean birthweight of infants resulting from embryos transferred on day 3 (3422 g ($\pm$ 547), *P* = 0.732). Moreover, newborn gestational age and infant gender were not significantly different between infants from day 3 transfer and those from day 5–6 transfer.

Table 3 shows the distribution of SGA and LGA infants according to maternal and treatment characteristics. The total proportions of SGA and LGA infants were 5.0 and 22.5%, respectively. Neither maternal (age and pattern of infertility) nor treatment characteristics (insemination method in a fresh

Fig. 1 Flow diagram of the study

cycle and stimulation protocol in a FET cycle) had any significant effect on the distribution of SGA/LGA infants. However, BMI significantly affected the distribution of LGA infants. In the BMI < 20 group, the proportion of LGA infants was 12.8%. In the $20 \leq \text{BMI} \leq 25$ group, the proportion of LGA infants was 23.2%. In the BMI > 25 group, the proportion of LGA infants was 32.3%. Thus, the proportion of LGA infants increased significantly with BMI. However, the proportion of

SGA infants was not significantly different among the three BMI groups. Regarding culture duration, the distributions of SGA and LGA newborns from Day 3 transfer were similar to those from day 5 to 6 transfer.

To further analyze the effect of culture duration on the birthweight of infants, we calculated the absolute and the gestational age- and gender-adjusted birthweights (z scores) of all infants. No significant difference was observed in the absolute mean birthweight between infants from day 3 and day 5–6 transfers (Table 4). The mean z score was 0.515, and no

Table 1 Main maternal and treatment characteristics of live-born singletons according to culture duration

	Day 3	Day 5–6	<i>P</i> value
Cycles/singletons (<i>n</i>)	554	538	–
Female age (years)	31.4 ± 4.4	30.4 ± 4.4	0.0003
BMI (kg/m ²)	22.4 ± 3.2	22.2 ± 3.1	0.228
Pattern of infertility			
Primary	317(57.2) ^a	298 (55.4)	0.542
Secondary	237 (42.8)	240 (44.6)	
Duration of infertility	4.2 ± 2.9	3.9 ± 2.7	0.130
Insemination method in fresh cycles			
IVF	420 (75.8)	452 (84.0)	0.001
ICSI	134 (24.2)	86 (16.0)	
Stimulation protocol in FET cycles			
hCG-induced natural cycles	145 (26.2)	149 (27.7)	0.571
HRT cycles	409 (73.8)	389 (72.3)	
Thickness of endometrium (mm)	9.6 ± 1.6	9.5 ± 1.5	0.226

BMI, body mass index; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; FET, frozen embryo transfer; HRT, hormone replacement therapy

^a Values in parenthesis are expressed in percentage

Table 2 Perinatal outcomes according to culture duration

	Day 3	Day 5–6	<i>P</i> value
Gestational age (weeks)	38.8 ± 1.6	38.8 ± 1.9	0.905
Normal, 37+ weeks	512 (92.4) ^a	490 (91.1)	0.486
Preterm, < 37 weeks	37 (6.7)	39 (7.2)	
Very preterm, < 32 weeks	5 (0.9)	9 (1.7)	
Infant gender			
Male	303 (54.7)	305 (56.7)	0.506
Female	251 (45.3)	233 (43.3)	
Mean birthweight (g)	3422 ± 547	3433 ± 559	0.732
Normal weight, 2500–4000 g	477 (86.1)	453 (84.2)	0.859
LBW (< 2500 g)	17 (3.1)	20 (3.7)	
VLBW (< 1500 g)	4 (0.7)	5 (0.9)	
HBW (> 4000 g)	45 (8.1)	51 (9.5)	
VHBW (> 4500 g)	11 (2.0)	9 (1.7)	

LBW, low birth weight; VLBW, very low birth weight; HBW, high birth weight; VHBW, very high birth weight

^a Values in parenthesis are expressed in percentage

Table 3 Distribution of small, average and large for gestational age (SGA, AGA, and LGA) infants according to maternal and treatment characteristics

	SGA	AGA	LGA
All	54 (5.0) ^a	792 (72.5)	246 (22.5)
Age			
< 35	44 (4.8)	667 (72.5)	209 (22.7)
> 35	10 (5.8)	125 (72.7)	37 (21.5)
BMI			
< 20	12 (5.7)	172 (81.5)	27 (12.8) ^b
20–25	37 (5.1)	516 (71.7)	167 (23.2) ^b
> 25	5 (3.1)	104 (64.6)	52 (32.3) ^b
Pattern of infertility			
Primary	29 (4.7)	442 (71.9)	144 (23.4)
Secondary	25 (5.2)	350 (73.4)	102 (21.4)
Insemination method in fresh cycles			
IVF	41 (4.7)	637 (73.1)	194 (22.2)
ICSI	13 (5.9)	155 (70.5)	52 (23.6)
Stimulation protocol in FET cycle			
hCG-induced natural cycles	11 (3.7)	229 (77.9)	54 (18.4)
HRT cycles	43 (5.4)	563 (70.6)	192 (24.1)
Culture duration			
Day 3	31 (5.6)	399 (72.0)	124 (22.4)
Day 5–6	23 (4.3)	393 (73.0)	122 (22.7)

BMI, body mass index; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; FET, frozen embryo transfer; HRT, hormone replacement therapy

^a Values in parenthesis are expressed in percentage

^b $P < 0.0001$

significant difference in z scores was found between the two groups (0.499 vs. 0.533, $P = 0.625$; Table 4).

We also performed a multiple linear regression analysis to determine the relationships between culture duration or other factors and singleton birthweight. As shown in Table 5, maternal BMI, infant gestational age, and infant gender significantly affected the singleton birthweight. However, culture duration did not significantly affect singleton birthweight ($P = 0.731$).

Table 4 Absolute and gestational age- and gender-adjusted mean birthweights (z scores) of singletons according to culture duration

	Total	Day 3	Day 5–6
n	1092	554	538
Mean birthweight (g) (SD)	3428 ± 553	3422 ± 547 ^a	3433 ± 559 ^a
Mean z score	0.515	0.499 ^b	0.533 ^b
95% CI	0.447–0.584	0.399–0.598	0.438–0.628

g, gram; SD, standard deviation; CI, confidence interval

^a $P = 0.732$ between Day 3 and Day 5–6 transfer

^b $P = 0.625$ between Day 3 and Day 5–6 transfer

Discussion

Our retrospective study shows that day 5–6 transfer in FET cycles did not increase the risks of preterm birth and LBW compared with day 3 transfer. No significant differences in HBW and VHBW were found between Day 5–6 transfer and Day 3 transfer (9.5 vs. 8.1% and 1.7 vs. 2.0%, respectively), which was similar to fresh cycles (8.9 and 1.5%, respectively) [18]. Moreover, no significant difference in the mean birthweight of live-born singletons was found between the two groups.

Culture duration has been reported to increase the proportion of LGA infants resulting from fresh embryo transfer [9, 10]. However, our retrospective study showed that culture duration did not significantly affect the proportion of LGA infants from FET cycles. In general, the proportion of LGA infants increased with maternal BMI. The proportion of LGA infants among mothers with a high BMI was almost three times higher than that among mothers with a low BMI, which is consistent with the findings from fresh cycles [9, 10]. Moreover, FET is a significant independent risk factor for LGA infants in IVF patients in addition to maternal BMI [20]. Several studies have reported that singleton birthweight and the proportion of LGA infants from FET cycles were significantly higher than those from fresh embryo transfer cycles [20–25]. In our retrospective study, the total proportion of LGA was 22.5%, which was higher than that for fresh cycles (9.4–11.4%) [9, 10, 20]. This finding may be associated with the exogenous gonadotrophin stimulation in fresh embryo transfer, which can affect the maternal uterine environment, leading to the limitation of fetal growth. Natural cycles have also been reported to be associated with an increased mean birthweight and a decreased incidence of low birthweight compared with stimulated cycles [26, 27]. FET cycles were analyzed in our retrospective study to avoid the influence of gonadotropin stimulation on the birthweight of newborns. However, imprinting diseases have been reported in humans and animals born via ART [28–30]. In animal studies, a higher incidence of large offspring syndrome was found among cattle and sheep conceived via ART [31]. The incidence of a similar epigenetic syndrome, Beckwith-Wiedemann syndrome (BWS), was significantly higher in children conceived via ART than that in children from spontaneous conception [32]. Therefore, the influence of epigenetic alteration at early embryonic stages during freezing and thawing procedures on the birthweight of newborns cannot be ignored, and the mechanism of epigenetic alteration requires further investigation.

Considering the influence of neonatal gestational age and gender on the birthweight of newborns, the z scores were used to evaluate the gender- and gestational age-adjusted birthweight of singletons. Two retrospective studies have shown that the z scores of singleton newborns from blastocyst

Table 5 Results of multiple linear regression analysis on the birthweight of live-born singletons

Model	Non-standardized coefficients		Standardized coefficients		Significance
	B	Standard error	Beta	t	
Constant	− 4295.096	345.779		− 12.421	< 0.0001
Culture duration (day 5–6 vs. day 3)	9.252	26.957	0.008	0.343	0.731
Female age (years)	− 0.380	3.529	− 0.003	− 0.108	0.914
BMI (kg/m ²)	25.721	4.367	0.145	5.891	< 0.0001
Pattern of infertility	− 14.184	28.788	− 0.013	− 0.493	0.622
Duration of infertility	− 8.486	5.369	− 0.043	− 1.581	0.114
Type of insemination in fresh cycles	− 5.626	34.114	− 0.004	− 0.165	0.869
Stimulation protocol in FET cycles	− 11.694	30.613	− 0.009	− 0.382	0.703
Thickness of endometrium	0.166	8.813	0.000	0.019	0.985
Gestational age (weeks)	187.194	7.707	0.591	24.289	< 0.0001
Infant gender (girls vs. boys)	− 139.792	26.897	− 0.126	− 5.197	< 0.0001

BMI, body mass index; FET, frozen embryo transfer

transfer were significantly higher than those of singleton newborns from embryo transfer in a fresh cycle [9, 10]. Another study showed that the *z* scores of singleton newborns from blastocyst transfer were significantly lower than those of singleton newborns from embryo transfer in FET cycles [14]. However, the above three studies were limited by a small sample size of blastocyst transfers in fresh or FET cycles (respectively including 69, 96 and 58 blastocyst transfers). According to the result of our study, which included a larger sample size of blastocyst transfers in FET cycles, the *z* scores of singleton newborns were not significantly affected by culture duration.

Several maternal confounding factors can affect the birthweight of singletons. Multiple linear regression analysis in our retrospective study showed that maternal BMI significantly affected the birthweight of singletons, which is consistent with the literature [12]. Maternal smoking was also a confounding factor affecting the birthweight of newborns. According to a report, approximately 2.4% of Chinese women smoke [10]. Data on maternal smoking, however, were not available in our database, which was a limitation of our retrospective study. De Vos et al. showed that maternal parity was significantly associated with singleton birthweight [12]. At the same time, data on this confounding factor were also not included in our database. We analyzed the influence of the pattern of infertility on singleton birthweight and found that secondary infertility was not a significant maternal confounding factor. Culture duration was also not a significant factor affecting the birthweight of singletons according to the results of multiple linear regression analysis. De Vos et al. [14] showed that blastocyst transfer in FET cycles significantly decreased the birthweight of singletons compared to embryo transfer. This conclusion may be attributed to the small sample size of that study.

Conclusions

Our retrospective study suggests that culture duration does not affect the birthweight of singletons resulting from embryo and blastocyst transfers in FET cycles. A large, multi-center randomized controlled trial (RCT) is needed to confirm our conclusion.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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