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The gestational age-specific difference in birthweight between singletons born after fresh and frozen embryo transfer: A cohort study

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Abstract

Introduction: Accumulating studies have suggested singletons born after frozen embryo transfer (FET) were higher than those born after fresh embryo transfer (Fre-ET). However, fewer studies had investigated the gestational age-specific between-group difference in birthweight. This study aimed to investigate the gestational week-specific difference in singleton birthweight after FET vs Fre-ET and explore potential factors that impact the difference.

Material and methods: In this retrospective cohort study, a total of 25 863 singletons were included. Multivariable linear regression and logistic regression were used to evaluate the between-group differences in mean birthweight and the incidences of large for gestational age (LGA) and small for gestational age (SGA), respectively.

Results: Multivariable regression analyses showed a statistically significant interaction between types of embryo transfer (ie FET vs Fre-ET) and the gestational week on mean birthweight ($P < 0.001$) and on the risks of large for gestational age ($P = 0.001$) and small for gestational age ($P < 0.001$). When stratified by gestational week, the differences in mean birthweight and the risks of LGA and SGA were only observed in singletons born at 37 gestational weeks or later. After adjusting for confounders, full-term but not preterm singletons born after FET had a higher birthweight (3497.58 ± 439.73 g vs 3445.67 ± 450.24 g; adjusted mean difference 58.35 g; 95% confidence interval [CI] 38.72–77.98 g), a higher risk of LGA (24.3% vs 21.1%; adjusted odds ratio [OR] 1.28, 95% CI 1.15–1.42) and a lower risk of SGA (3.1% vs 4.8%; adjusted OR 0.61, 95% CI 0.53–0.70) compared with those born after Fre-ET.

Conclusions: The differences in birthweight between FET and Fre-ET were observed in full-term singletons but not preterm singletons.

Abbreviations: AC, artificial cycle regimen; MD, mean difference; OR, odds ratio; CI, confidence interval; FET, frozen embryo transfer; Fre-ET, fresh embryo transfer; GW, gestational week; hCG, human chorionic gonadotropin; LGA, large for gestational age; NC, natural cycle regimen; SGA, small for gestational age.

KEYWORDS
birthweight, fresh embryo transfer, frozen embryo transfer, large for gestational age, small for gestational age

1 | INTRODUCTION

The global application of frozen embryo transfer (FET) has been increasing over the last decades. Although the maternal and fetal outcomes after FET were largely reassuring,[1,2] accumulating evidence suggested that singletons born after FET had a higher birthweight and a higher risk of being born large for gestational age (LGA) compared with babies born after fresh embryo transfer (Fre-ET).[2–5] When compared with spontaneously conceived babies, babies from FET still had higher risks of being LGA and of macrosomia.[6] The underlying mechanism for the increased birthweight after FET is still not fully understood. FET cycles could avoid the adverse effect on embryo implantation and placentation by the supraphysiologic estrogenic environment during Fre-ET, which was suggested to exert a negative impact on birthweight.[7–9] The freezing/thawing procedures might cause epigenetic alterations which were critical for fetal growth.[10–12] Furthermore, the regimens for endometrial preparation during FET cycles were also found to affect birthweight.[13–15]

However, the implications of the increase in birthweight after FET on offspring short-term or long-term health are unclear. Birthweight is an important predictor for the baby's survival and is closely related to health later in life.[16] The association between birthweight and the health of babies varied with the gestational week at birth.[17–19] Among prematurely delivered babies, larger birthweight was a predictor for decreased risks of neonatal mortality and morbidities.[17,18] However, among term babies, the increase in birthweight was associated with higher risks of composite maternal and neonatal complications.[19] Both high birthweight and low birthweight are associated with long-term risk for cardiovascular and metabolic disease.[20–22]

Several studies had explored the fetal growth trajectories during pregnancies after FET and Fre-ET and have reported controversial results.[23–25] It was shown that the between-group difference in growth kinetics began in the second trimester,[23] or even as early as the first trimester.[24,25] Meanwhile, beyond fetal growth kinetics, birthweight was closely related to the length of pregnancy before delivery.[26] Fewer studies had investigated the gestational age-specific difference in birthweight between FET and Fre-ET. In this retrospective cohort study, we evaluated the discrepancies in birthweight between Fre-ET and FET, considering the gestational age at birth, and explored the factors modifying the association.

Key message

Statistically significant differences in birthweight between frozen embryo transfer and fresh embryo transfer were found at 37 gestational weeks at birth or later. The between-group differences seemed to occur earlier in women with a higher estradiol level on the day of hCG trigger.

2 | MATERIAL AND METHODS

This study enrolled women who underwent their first in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles at the Center for Reproductive Medicine affiliated to Shandong University from January 2012 to March 2020. Women aged 20–40 years and those who achieved a singleton live birth (defined as deliveries with a live baby at 28 weeks' gestation or later) were included in the analysis. If the women had more than one delivery, only the first live birth was included. We excluded pregnancies after oocyte donation, oocyte cryopreservation, in vitro maturation of oocyte, preimplantation genetic testing and vanishing twins. Women with polycystic ovary syndrome (PCOS) diagnosed by the Rotterdam criteria were also excluded.[27]

Conventional IVF and ICSI were performed as previously described.[28,29] For the Fre-ET group, embryo transfer was performed on Day 3 or Day 5 of embryo culture at the discretion of physicians. Up to two embryos were transferred. Luteal phase support was started on the day of oocyte retrieval and continued till 11 weeks of gestation.

In women who underwent FET, endometrial preparation was achieved by a natural cycle regimen (NC-FET) or an artificial cycle regimen (AC-FET) at the discretion of physicians. For the natural cycle regimen, the timing of embryo transfer was determined by the day of ovulation. Cleavage-stage embryos were transferred on Day 3 after ovulation and blastocysts were transferred on Day 5 after ovulation. Luteal phase support was started on the day of ovulation and ceased at 11 weeks of gestation. For the artificial cycle regimen, oral estrogen (Progynova, Delpharm Lille) was administered to prime endometrium from Day 2 or Day 3 of the menstrual cycle. Progesterone was added when the endometrial thickness reached at least 7 mm. Cleavage-stage embryos were transferred on Day 3 after progesterone administration and blastocysts were transferred on Day 5 after progesterone administration. If conception occurred after FET, estrogen was continued until 8 weeks of gestation, and progesterone was continued until 11 weeks of gestation.

Afterwards, for women who achieved viable pregnancies, trained nurses carried out telephone follow-ups at the time of delivery and recorded the obstetric complications, neonatal gender, gestational week (GW) at birth, and birthweight. GW was calculated based on the date of embryo transfer and the stage of embryo transfer.

The main outcomes were mean birthweight, LGA and small for gestational age (SGA). LGA and SGA were defined as birthweight higher than the 90th percentile and lower than the 10th percentile based on the Chinese reference for birthweight, respectively.[30]

2.1 | Statistical analyses

Categorical variables were expressed as the number of cases (*n*) with the percentage of occurrence (%) and continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile range). Chi-square test or Fisher's exact tests were used to compare categorical variables, while Student's *t*-test and Wilcoxon rank-sum test were used to evaluate the continuous variables, as appropriate. The Shapiro–Wilk normality test and visual inspection of the distributions were also used to check for normality.

Multivariable linear regression analysis was performed to assess the association between types of embryo transfer and mean birthweight, and logistic regression analysis was used to evaluate the effect of types of embryo transfer on the incidences of LGA and SGA. The same set of potential confounders was introduced into the regression models, including maternal age (categorical), body mass index (BMI, categorical), number of oocytes retrieved (continuous), progesterone level on the day of human chorionic gonadotropin (hCG) trigger (continuous), number of oocytes transferred (categorical), stage of embryo transferred (categorical), infertility diagnosis (categorical), methods of fertilization (categorical), parity (0 or ≥1), chronic hypertension (categorical), pregestational diabetes (categorical), infant gender (categorical), GW at birth (continuous), and the interaction between types of embryo transfer and GW at birth. To present the results clearly, we stratified the GW at birth into preterm birth (<37 weeks), full-term birth (at least 37 weeks but <41 weeks) and post-term birth strata (at least 41 weeks).[15] Using the Fre-ET group as reference, adjusted mean difference (MD) of birthweight, adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated.

To explore the factors modifying the association between types of embryo transfer and birthweight, we performed subgroup analyses based on the level of the estradiol on the day of hCG trigger. Furthermore, among singletons born after FET, we compared the birthweight between different regimens used for endometrial preparation, ie AC-FET and NC-FET. Statistical significance was set at $P < 0.05$ and all data analyses were performed using SPSS (SPSS Inc., Version 21.0).

2.2 | Ethics statement

The study protocol was approved by the Ethics Committees of the Center for Reproductive Medicine affiliated to Shandong University on January 13, 2022 (2022–03). Each patient signed an informed consent to use their de-identified clinical data for research at the initiation of treatment.

3 | RESULTS

A total of 25 863 singletons, 13 542 from FET and 12 321 from Fre-ET, were enrolled (Table 1). Maternal age (30.15 ± 4.04 vs 30.71 ± 4.15, $P < 0.001$) and BMI (23.04 ± 3.42 vs 23.31 ± 3.50, $P < 0.001$) were lower in the FET group than in the Fre-ET group. Women in the FET group had a higher oocyte yield (14.33 ± 6.12 vs 10.11 ± 4.69, $P < 0.001$) and a higher proportion of blastocyst transfer (98.4% vs 20.4%, $P < 0.001$) than those in the Fre-ET group. Compared with the Fre-ET group, the GW at birth was comparable, but the percentages of post-term birth (7.8% vs 7.1%; $P = 0.038$) and preterm birth (6.0% vs 5.4%; $P = 0.033$) were higher in the FET group. Women who underwent FET were more likely to have had hypertensive disorders in pregnancy (4.5% vs 3.0%, $P < 0.001$), gestational diabetes mellitus (6.7% vs 6.0%, $P = 0.018$) and cesarean section (71.1% vs 66.9%, $P < 0.001$) compared with those who underwent Fre-ET.

Multivariable regression analyses showed a statistically significant interaction between types of embryo transfer (ie FET vs Fre-ET) and GW at birth on mean birthweight ($P < 0.001$) and on the incidences of LGA ($P = 0.001$) and SGA ($P < 0.001$; Table 2). The statistically significant differences in mean birthweight between singletons born from FET and those from Fre-ET were only observed in babies born at GW 37 or later (Table 3). The incidences of LGA increased with GW and the incidences of SGA decreased with GW both in the Fre-ET and FET groups (Table 4). Compared with singletons born after Fre-ET, babies from FET had significantly higher proportions of LGA starting from GW 38 and had significantly lower proportions of SGA starting from GW 37 to GW40 (Table 4).

The mean birthweight of LGA was >4000 g, which was approximately 700 g heavier than those of appropriate for gestational age. The rate of cesarean section among pregnancies with LGA was about 80% (Table S1).

After adjusting for confounders, singletons after FET had a higher birthweight in the full-term subgroup (3497.58 ± 439.73 g vs 3445.67 ± 450.24 g; adjusted MD 58.35 g; 95% CI 38.72–77.98 g) and post-term subgroup (3675.49 ± 416.41 g vs 3631.00 ± 422.21 g; adjusted MD 55.27 g; 95% CI 18.24–92.29 g), a higher risk of LGA in both the full-term subgroup (24.3% vs 21.1%; adjusted OR 1.28, 95% CI 1.15–1.42) and the post-term subgroup (27.4% vs 22.9%; adjusted OR 1.63, 95% CI 1.20–2.20) and a lower risk of SGA in the full-term subgroup (3.1% vs 4.8%; adjusted OR 0.61, 95% CI 0.53–0.70) compared with those born after Fre-ET (Table 2).

In the subgroup analysis by estradiol level on the day of hCG trigger, the study population was divided into the lower estradiol subgroup (7999 births after Fre-ET and 4999 births after FET) and higher estradiol subgroup (4322 births after Fre-ET and 8543 births after FET) according to the median of estradiol levels (3454 pg/mL). In the lower estradiol subgroup, compared with singletons born from Fre-ET, singletons born from FET had a higher incidence of LGA starting from GW 39, and a lower incidence of SGA starting from GW 37 to GW 39 (Table 5). In the higher estradiol subgroup, compared with singletons born from Fre-ET, singletons born from FET had a higher incidence of LGA starting from GW 37 to GW 39, and a significantly lower proportion of SGA starting from GW 36 to GW 39 (Table 5).

Among infants born after FET, we found a statistically significant interaction between different regimens for endometrial preparation and GW on birthweight ($P = 0.017$) and the incidence of LGA ($P = 0.026$; Table 6). After adjustment for confounding factors, compared with singletons born after NC-FET, full-term singletons born after AC-FET had a higher birthweight (3524.90 ± 456.31 vs

TABLE 1 Maternal characteristics and perinatal outcomes of singleton pregnancies after frozen embryo transfer and fresh embryo transfer

	FET (n = 13 542)		Fre-ET (n = 12 321)		
	n	%, or mean ± SD, or median (interquartile range)	n	%, mean ± SD, or median (interquartile range)	P-value
Maternal age (years)		30.15 ± 4.04		30.71 ± 4.15	<0.001
<25	907	6.7	729	5.9	<0.001
25 ~ 30	5540	40.9	4381	35.5	
30 ~ 35	4940	36.5	4787	38.9	
≥35	2155	15.9	2424	19.7	
Body mass index (kg/m^2)		23.04 ± 3.42		23.31 ± 3.50	<0.001
<25	10 085	74.5	8809	71.5	<0.001
≥25	3457	25.5	3512	28.5	
Infertility diagnosis					0.002
Male factor	1311	9.7	1159	9.4	
Pelvic factors	10 220	75.5	9121	74.0	
Pelvic and male factor	1387	10.2	1428	11.6	
Others	624	4.6	613	5.0	
Parity≥1	2574	19.0	2727	22.1	<0.001
Pregestational diabetes	39	0.3	37	0.3	0.855
Chronic hypertension	599	4.4	623	5.1	0.017
Number of oocytes retrieved		14.33 ± 6.12		10.11 ± 4.69	<0.001
Methods of fertilization					0.409
IVF	9874	72.9	9055	73.5	
ICSI	3260	24.1	2882	23.4	
Half IVF/half ICSI	408	3.0	384	3.1	
Cycles using donor sperms	1144	8.4	1029	8.4	0.781
Progesterone level on the day of hCG trigger (ng/mL)		0.89 (0.65,1.18)		0.77 (0.57, 1.02)	<0.001
Estradiol level on the day of hCG trigger (pg/mL)		4628.52 ± 2348.77		3158.22 ± 1639.38	<0.001
Number of embryos transferred					<0.001
One embryo	11 853	87.5	3186	25.9	
Two embryos	1689	12.5	9135	74.1	
Stage of embryo transferred					<0.001
Cleavage stage	211	1.6	9813	79.6	
Blastocyst stage	13 331	98.4	2508	20.4	
GDM	907	6.7	737	6.0	0.018
HDP	603	4.5	372	3.0	<0.001
Cesarean section[a]	9634	71.1	8243	66.9	<0.001
Gestational weeks at birth		39.21 ± 1.58		39.24 ± 1.48	0.094
Preterm	818	6.0	668	5.4	0.033
Full-term	11 674	86.2	10 781	87.5	0.002
Post-term	1050	7.8	872	7.1	0.038
Infant gender					0.001
Female	6325	46.7	5999	48.7	
Male	7217	53.3	6322	51.3	

Note: Unless otherwise stated, values are expressed as mean ± SD, median (interquartile range) or n (%). A P-value <0.05 was considered statistically significant.

Abbreviations: FET, frozen embryo transfer; Fre-ET, fresh embryo transfer; GDM, gestational diabetes mellitus; hCG, human chorionic gonadotropin; HDP, hypertensive disorders in pregnancy; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization.

[a]Five values in the FET group and one value in the Fre-ET group were missed.

TABLE 2 Comparison of birthweight between singletons after frozen embryo transfer and fresh embryo transfer

	FET (n = 13542)		Fre-ET (n = 12321)		Adjusted MD or OR			
	n	%, or mean ± SD	n	%, or mean ± SD	MD/OR	95% CI	P-value	P-value for interaction between types of embryo transfer and GW
Birthweight (g)[a]		3453.86 ± 510.30		3410.73 ± 507.27	−59.90	−325.11 to 205.32	0.658	<0.001
Preterm[b]		2545.38 ± 643.30		2559.25 ± 659.27	0.36	−109.63 to 110.35	0.995	N/A
Full-term[b]		3497.58 ± 439.73		3445.67 ± 450.24	58.35	38.72–77.98	<0.001	N/A
Post-term[b]		3675.49 ± 416.41		3631.00 ± 422.21	55.27	18.24–92.29	0.003	N/A
LGA[a]	3227	23.8	2561	20.8	1.06	0.52–2.18	0.870	0.001
Preterm[b]	102	12.5	83	12.4	1.03	0.62–1.72	0.909	N/A
Full-term[b]	2837	24.3	2278	21.1	1.28	1.15–1.42	<0.001	N/A
Post-term[b]	288	27.4	200	22.9	1.63	1.20–2.20	0.002	N/A
SGA[a]	451	3.3	604	4.9	0.75	0.39–1.42	0.376	<0.001
Preterm[b]	67	8.2	69	10.3	1.16	0.60–2.26	0.663	N/A
Full-term[b]	357	3.1	513	4.8	0.61	0.53–0.70	<0.001	N/A
Post-term[b]	27	2.6	22	2.5	0.64	0.24–1.70	0.369	N/A

Note: Unless otherwise stated, values are expressed as mean ± SD or n (%). A P-value <0.05 was considered statistically significant.

Abbreviations: CI, confidence interval; FET, frozen embryo transfer; Fre-ET, fresh embryo transfer; GW, gestational week; LGA, large for gestational age; MD, mean difference; N/A, not applicable; OR, odds ratio; SGA, small for gestational age.

[a]Adjustments included maternal age (category), body mass index (category), number of transferred embryos, stage of embryo transfer, infertility diagnosis, methods of fertilization, parity, chronic hypertension, pregestational diabetes, progesterone level on the day of hCG trigger, number of oocytes retrieved, infant gender, GW and the interaction between the types of embryo transfer and GW.

[b]Adjustments included maternal age (category), body mass index (category), number of transferred embryos, stage of embryo transfer, infertility diagnosis, methods of fertilization, parity, chronic hypertension, pregestational diabetes, progesterone level on the day of hCG trigger, number of oocytes retrieved and infant gender.

TABLE 3 Mean birthweight between singletons after frozen embryo transfer and fresh embryo transfer for each gestational week at birth

	FET birthweight (g) (n = 13 542)		Fre-ET birthweight (g) (n = 12 321)		
GW	n	Mean ± SD	n	Mean ± SD	P-value
≤33	211	1856.94 ± 539.58	152	1857.17 ± 581.26	0.997
34	98	2485.10 ± 545.58	83	2400.18 ± 443.50	0.258
35	176	2715.77 ± 429.14	145	2644.42 ± 499.37	0.170
36	333	2909.28 ± 446.86	288	2932.76 ± 488.88	0.532
37	936	3216.67 ± 450.27	792	3154.21 ± 497.04	0.006
38	2732	3416.88 ± 435.93	2448	3354.53 ± 455.75	<0.001
39	4675	3523.34 ± 425.43	4542	3472.54 ± 425.41	<0.001
40	3331	3606.57 ± 413.12	2999	3556.35 ± 421.30	<0.001
≥41	1050	3675.49 ± 416.41	872	3631.00 ± 422.21	0.021

Note: Unless otherwise stated, values are expressed as mean ± SD. A P-value <0.05 was considered statistically significant.

Abbreviations: FET, frozen embryo transfer; Fre-ET, fresh embryo transfer; GW, gestational week.

TABLE 4 Proportions of LGA and SGA between singletons after frozen embryo transfer and fresh embryo transfer for each gestational week at birth

	LGA FET (n = 3227)		LGA Fre-ET (n = 2561)			SGA FET (n = 451)		SGA Fre-ET (n = 604)		
GW	n	%	n	%	P-value	n	%	n	%	P-value
≤33	21	10.0	14	9.2	0.813	24	11.4	22	14.5	0.381
34	15	15.3	8	9.6	0.254	11	11.2	9	10.8	0.935
35	27	15.3	19	13.1	0.569	12	6.8	15	10.3	0.257
36	39	11.7	42	14.6	0.289	20	6.0	23	8.0	0.332
37	166	17.7	119	15.0	0.130	35	3.7	69	8.7	<0.001
38	653	23.9	515	21.0	0.014	93	3.4	154	6.3	<0.001
39	1137	24.3	943	20.8	<0.001	132	2.8	175	3.9	0.006
40	881	26.4	701	23.4	0.005	97	2.9	115	3.8	0.042
≥41	288	27.4	200	22.9	0.024	27	2.6	22	2.5	0.946

Note: Unless otherwise stated, values are expressed as n (%). A P-value <0.05 was considered statistically significant.

Abbreviations: FET, frozen embryo transfer; Fre-ET, fresh embryo transfer; GW, gestational week; LGA, large for gestational age; SGA, small for gestational age.

3484.56 ± 431.03 g; adjusted MD 25.66 g; 95% CI 8.93–42.40 g) and a higher risk of LGA (26.7% vs 23.2%; adjusted OR 1.14, 95% CI 1.04–1.24; Table 6).

4 | DISCUSSION

This cohort study showed that the difference in birthweight between FET and Fre-ET varied with gestational age at birth. The statistically significant between-group differences in birthweight and the incidence of LGA were only observed in full-term singletons and post-term singletons, and not in preterm singletons. Pregnancies with LAG were associated with a higher rate of cesarean section, which was about 80%. The between-group differences seemed to occur earlier in women with a higher estradiol level than in those with a lower estradiol level on the day of hCG trigger. Among infants born from FET, compared with NC-FET, AC-FET was associated with a higher birthweight in full-term singletons.

Many studies have compared perinatal outcomes between FET and Fre-ET,[2–5] but fewer have investigated the between-group difference in birthweight stratified by GW at birth. In a large Nordic population-based cohort study,[31] Terho and colleagues found the between-group differences in birthweight started from GW 33. Intrauterine growth curves based on ultrasound examination have been considered a better indication of fetal growth. Ginod et al.[23] compared crown–rump length and estimated fetal weight among pregnancies resulting from Fre-ET, intrauterine insemination and FET, and found growth kinetics between different assisted reproductive technologies differed from the second trimester of pregnancy. Compared with these studies, the statistically significant difference in birthweight between FET and Fre-ET was only found in full-term singletons in our study. The reasons for the discrepancies between studies are unknown. The estradiol level during Fre-ET was not reported in those studies[23,31] and all FET used AC for endometrial preparation in the study by Ginod et al.[23] Whether those differences contributed to the discrepancies is unknown.

TABLE 5 Proportions of LGA and SGA between singletons after frozen embryo transfer and fresh embryo transfer for each gestational week at birth stratified by estradiol level on the day of hCG trigger

	Lower estradiol subgroup									Higher estradiol subgroup								
	LGA FET $n = 1284$		LGA Fre-ET $n = 1766$			SGA FET $n = 141$		SGA Fre-ET $n = 349$			LGA FET $n = 1943$		LGA Fre-ET $n = 795$			SGA FET $n = 310$		SGA Fre-ET $n = 255$		
GW	*n*	%	*n*	%	*P*-value	*n*	%	*n*	%	*P*-value	*n*	%	*n*	%	*P*-value	*n*	%	*n*	%	*P*-value
≤33	5	6.5	11	10.4	0.358	9	11.7	15	14.2	0.626	16	11.9	3	6.5	0.409	15	11.2	7	15.2	0.472
34	7	17.5	6	12.0	0.461	2	5.0	5	10.0	0.456	8	13.8	2	6.1	0.318	9	15.5	4	12.1	0.763
35	9	11.0	11	10.9	0.985	5	6.1	7	6.9	0.821	18	19.1	8	18.2	0.892	7	7.4	8	18.2	0.078
36	16	11.3	31	16.4	0.194	10	7.1	10	5.3	0.498	23	12.0	11	11.1	0.827	10	5.2	13	13.1	0.018
37	78	21.6	90	17.8	0.156	10	2.8	40	7.9	0.001	88	15.3	29	10.2	0.039	25	4.3	29	10.2	0.001
38	275	25.5	371	22.9	0.126	29	2.7	94	5.8	<0.001	378	22.9	144	17.4	0.002	64	3.9	60	7.2	<0.001
39	440	26.0	661	22.5	0.006	38	2.2	98	3.3	0.035	697	23.4	282	17.6	<0.001	94	3.2	77	4.8	0.005
40	347	29.5	456	24.0	0.001	32	2.7	68	3.6	0.192	534	24.8	245	22.4	0.122	65	3.0	47	4.3	0.061
≥41	107	30.7	129	22.2	0.004	6	1.7	12	2.1	0.711	181	25.8	71	24.4	0.640	21	3.0	10	3.4	0.716

Note: Unless otherwise stated, values are expressed as *n* (%). A *P*-value <0.05 was considered statistically significant.

Abbreviations: FET, frozen embryo transfer; Fre-ET, fresh embryo transfer; GW, gestational week; LGA, large for gestational age; SGA, small for gestational age.

Nevertheless, the consistency among our study and theirs is that the between-group difference in birthweight after frozen vs fresh embryo transfer become even larger with the progress of pregnancy. The association between birthweight and neonatal prognosis varied with GW at birth. Being born LGA was a better prognosis for premature newborns[17,18] but not for full-term newborns.[19] The increased risk of LGA in full-term babies born after FET suggests that a close follow-up of their long-term health is warranted.

Furthermore, our data enabled us to perform subgroup analyses to explore the factors affecting the between-group difference in birthweight. In the subgroup with higher estradiol levels on the day of hCG trigger, the difference in birthweight between FET and Fre-ET occurred earlier than the subgroup with lower estradiol levels. Our results suggested that the supraphysiological estradiol environment after ovarian stimulation might play a role in the decrease in birthweight and the increased between-group difference. This finding was supported by the studies which showed that serum estradiol level during ovarian stimulation was associated with elevated risks of LBW and SGA after Fre-ET.[7–9] However, the underlying mechanism between a high level of estradiol and lower birthweight is still not fully understood. Sustained higher concentrations of estradiol after ovarian stimulation can last up to 8 weeks of gestation after Fre-ET.[32] Previous studies showed that a higher level of estradiol could affect the development of gametes,[33] morphological and functional differentiation of villous trophoblast and endometrium,[34,35] as well as intrauterine fetal metabolism.[36] Animal studies suggested that exposure to a high estradiol environment in early pregnancy was associated with elevated expression of insulin-like growth factor binding protein 1 (IGFBP1) in the placenta and fetal liver tissues in the late pregnancy.[36] Insulin-like growth factor (IGFs, ie IGF-1and IGF-2) plays an important role in fetal metabolism, growth and development, where IGFBP1 could bind with IGFs and restrict the biological activities of free IGFs.[37] In the third trimester of pregnancy, fetal growth is the fastest and the fetus gains most of its weight.[38] In the late pregnancy, babies from Fre-ET may grow slower than those from FET, with a decrease in placental perfusion and an increased expression of IGFBP1. Future studies are needed to elucidate the underlying mechanism. The implications of this finding on clinical practice are that, if possible, we should avoid excess ovarian stimulation, and mild stimulation may be safer for both mothers and fetuses. On the other hand, in the case of high ovarian response, freezing all embryos and performing frozen embryo transfer may be more a better option.

With the progress of pregnancy, the incidence of LGA increased, whereas the incidence of SGA decreased, both gradually deviating from 10%. Based on the definitions of LGA and SGA, the incidence of LGA or SGA is 10% among natural conception for each GW at birth. Thus, the distribution of singleton birthweight in FET and Fre-ET deviated compared with that in natural conception. Embryonic development is susceptible to the external environment. IVF-ET process involves ovarian stimulation, gamete manipulation, embryo culture, as well as embryo freezing and thawing, which may induce epigenetic alteration.[12,39,40] The heterogeneity

TABLE 6 Comparison of birthweight between singletons after nature cycle regimen FET (NC-FET) and artificial cycle regimen FET (AC-FET)

	NC-FET (n = 9021)		AC-FET (n = 4521)		Adjusted MD or OR			
	n	%, or mean ± SD	n	%, or mean ± SD	MD/OR	95% CI	P-value	P-value for interaction between regimens for endometrial preparation and GW
Birthweight (g)[a]		3446.16 ± 494.02		3469.22 ± 541.05	−119.28	−475.06-236.51	0.511	0.017
Preterm[b]		2541.58 ± 630.84		2550.88 ± 661.85	4.16	−86.41 to 94.72	0.928	N/A
Full-term[b]		3484.56 ± 431.03		3524.90 ± 456.31	25.66	8.93–42.40	0.003	N/A
Post-term[b]		3658.64 ± 406.99		3700.97 ± 429.51	26.52	−24.09to 77.12	0.304	N/A
LGA[a]	2058	22.8	1169	25.9	0.77	0.30–1.97	0.590	0.026
Preterm[b]	60	12.4	42	12.6	0.95	0.61–1.47	0.815	N/A
Full-term[b]	1832	23.2	1005	26.7	1.14	1.04–1.24	0.006	N/A
Post-term[b]	166	26.3	122	29.2	1.12	0.84–1.49	0.453	N/A
SGA[a]	297	3.3	154	3.4	0.65	0.26–1.60	0.343	0.218
Preterm[b]	41	8.5	26	7.8	0.91	0.54–1.56	0.740	N/A
Full-term[b]	243	3.1	114	3.0	1.04	0.83–1.30	0.758	N/A
Post-term[b]	13	2.1	14	3.3	1.82	0.84–3.96	0.131	N/A

Note: Unless otherwise stated, values are expressed as mean ± SD or n (%). A P-value <0.05 was considered statistically significant.

Abbreviations: CI, confidence interval; FET, frozen embryo transfer; Fre-ET, fresh embryo transfer; GW, gestational week; LGA, large for gestational age; MD, mean difference; N/A, not applicable; OR, odds ratio; SGA, small for gestational age.

[a]Adjustments included maternal age (category), body mass index (category), number of embryos transferred, stage of embryo transferred, infertility diagnosis, methods of fertilization, parity, chronic hypertension, pregestational diabetes, progesterone level on the day of hCG trigger, number of oocytes retrieved, infant gender, GW and the interaction between regimens for endometrial preparation and GW.

[b]Adjustments included maternal age (category), body mass index (category), number of embryos transferred, stage of embryo transferred, infertility diagnosis, methods of fertilization, parity, chronic hypertension, pregestational diabetes, progesterone level on the day of hCG trigger, number of oocytes retrieved, infant gender.

between infertile and fertile populations may also contribute to the difference in birthweight.[41,42] Compared with fertile populations, subfertile patients who had a singleton birth without assisted reproductive technology were still at increased risk of several adverse outcomes, including LBW.[43]

Moreover, the regimens for endometrial preparation during FET cycles were found to affect birthweight as well, and AC-FET was associated with a higher birthweight and a higher risk of macrosomia compared with NC-FET.[13–15] In the present study, we found full-term but not preterm singleton birthweight was higher in the AC-FET group than that in the NC-FET group. In line with our results, Ishii and colleagues found that the birthweight of singletons from AC-FET was higher than those from NC-FET from 37 weeks of gestation.[44] These findings suggested that the natural ovulation cycle may be superior to an artificial cycle for endometrial preparation before FET in ovulatory women regarding birthweight. The underlying mechanisms for the increased birthweight after AC-FET vs NC-FET during late pregnancy are not fully understood. Nakamura et al. found a difference in placental basal plate between placentas from AC-FET and NC-FET.[45] In addition, previous studies have shown that the absence of corpus luteum during AC-FET may impact obstetric and perinatal outcomes.[46,47] The corpus luteum produces many hormones which are crucial for implantation, placentation and pregnancy maintenance.[48] Whether corpus luteum affects the growth trajectory of fetus requires further study.

The main strengths of the study lay in the large sample size and the detailed data on pre-pregnant conditions and each step of IVF, which enabled us to perform multivariable regression analysis and subgroup analysis. In addition, only patients who underwent their first IVF cycle were included, which decreased the risk of bias from multiple failed IVF cycles. To optimize the homogeneity of the included patients, we excluded patients with PCOS and vanishing twins, which may augment the risk of LGA[49,50] and SGA,[51] respectively.

There are also limitations to this study. First, as a retrospective cohort study, we cannot rule out the possible effect of bias and confounders. Secondly, though the sample size was more than 25 000, the number of premature newborns was still relatively small and meant that the analysis was underpowered to detect a statistically significant difference in preterm birthweight.

5 | CONCLUSION

In this study, we found a difference in birthweight between Fre-ET and FET in full-term singletons but not in preterm singletons. The between-group difference in birthweight seemed to occur earlier in women with a higher estradiol level on the day of hCG trigger. Among babies born from FET, AC-FET was associated with a higher birthweight in full-term babies compared with NC-FET. Further studies with larger samples are necessary to confirm our findings.

AUTHOR CONTRIBUTION

DMW and YHY contributed to the conception and design. QD and YN collected the data. QD performed data collation and statistical analyses. YHW, LS, YN and DYZ revised the data and contributed to the interpretation of the results. QD drafted the article, and DMW and YHY revised it critically for important intellectual content. All the authors read and approved the final version of the article.

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CONFLICT OF INTERESTS

None.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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