Effect of in vitro culture period on birth weight after vitrified-warmed transfer cycles: analysis of 4,201 singleton newborns

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Objective: To investigate the effect of embryo culture duration on birth weight in vitrified-warmed cycles.
Design: Retrospective cohort study.
Setting: Tertiary-care academic medical center.
Patient(s): A total of 4,201 women who gave birth to 3,520, 215, and 466 live-born singletons after frozen-thawed cleavage-stage (day 3) and day 5 and day 6 blastocyst transfer, respectively.
Intervention(s): None.
Main Outcome Measure(s): Neonatal birth weight.
Result(s): The mean birth weight did not differ between the three study groups. However, the gestational age– and sex-adjusted birth weight (*Z*-scores) of singletons and the proportion of large-for-gestational-age (LGA) babies were significantly higher after day 5 and day 6 transfer than after transfer of day 3 embryos. Furthermore, multiple linear regression analysis indicated that gestational age, parental body mass index, neonatal sex, and length of the culture period all had significant and strong impacts on birth weight of singleton newborns.
Conclusion(s): In the vitrified-warmed transfer cycles, birth weight *Z*-scores and the proportion of LGA infants were both higher in singletons born after blastocyst transfer than after transfer of cleavage-stage embryos. This finding suggests that the effect of culture duration was not overcome by transfer of embryos into a more physiologic uterine environment. (Fertil Steril® 2019;111: 97–104. ©2018 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Birth weight, vitrification, blastocyst transfer, cleavage-stage transfer

Refinement in cryopreservation technique and widespread adoption of single-embryo transfer facilitate the increasing use of frozen-embryo transfer (FET) globally. In the United States, the proportion of FET cycles versus fresh cycles rose from 15.3% in 2003 to 30.1% in 2015, resulting in an estimated 28,800 infants born in 2015 (1).

Received July 10, 2018; revised September 6, 2018; accepted October 3, 2018; published online November 17, 2018.
J.Z. has nothing to disclose. Y.W. has nothing to disclose. H.L. has nothing to disclose. X.M. has nothing to disclose. Q.C. has nothing to disclose. Y.F. has nothing to disclose. Y.X. has nothing to disclose. Y.K. has nothing to disclose.
Supported by the National Key Research and Development Program of China (grant no. SQ2018YFC100163), National Natural Science Foundation of China (grant nos. 81771533, 81571397, 31770989, and 81671520), and China Postdoctoral Science Foundation (grant no. 2018M630456).
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Fertility and Sterility® Vol. 111, No. 1, January 2019 0015-0282/$36.00

https://doi.org/10.1016/j.fertnstert.2018.10.006

Long-term safety of progeny after in vitro fertilization (IVF) is a major concern for couples seeking infertility treatment. Emerging evidence has suggested that babies resulting from FET have perinatal outcomes similar to or even better than peers born after fresh transfer cycles (2, 3). Nonetheless, these optimal FET outcomes did not persist when compared with those conceived naturally. Instead, the risk of adverse perinatal outcomes, especially regarding birth weight, is elevated in live-born singletons after FET (4, 5). Abnormal birth weight not only increases the risk of complications during the fetal and

neonatal periods, but also is a strong predictor of subsequent adulthood metabolic disease (6, 7).

Parental characteristics or subfertility per se may be, at least in part, responsible for abnormal neonatal birth weights (8). Furthermore, it has been speculated that in vitro embryo culture, both the composition of culture medium and culture duration, could be involved as well (9).

Existing data concerning the effect of culture duration on infant birth weight are conflicting. A number of studies documented higher mean birth weights and an increased proportion of large-for-gestational-age (LGA) neonates originating from blastocyst transfer compared with those born after day 3 embryo transfer (10–12), although others declare that no relationship between prolonged embryo culture and birth weight was observed (13–15). Nonetheless, these studies mainly focused on fresh transfer cycles, without ruling out the possibility of adverse fetal growth caused by a hyperestrogenic milieu (16). Whether or not the effect of culture duration could be overcome by cryopreservation remains inconclusive. A more recent study reported a significantly lower singleton birth weight *Z*-score after vitrified-warmed day 5 embryo transfer compared with cleavage-stage transfer, a difference not found in fresh transfer cycles (17). However, that study was limited by an exceedingly small sample size. With the advent of a freeze-all policy, exploration of the influence of culture duration on birth weight generated from vitrified-warmed cycles is of vital importance.

Therefore, the aim of the present study was to compare the birth weights of singletons after vitrified-warmed cycles with embryo culture to either cleavage stage or blastocyst.

MATERIALS AND METHODS

Study Design and Patients

A retrospective study was conducted at the Department of Assisted Reproduction of the Ninth People's Hospital of Shanghai Jiao Tong University School of Medicine. Women underwent FET cycles during the period from January 2013 to December 2016. Inclusion criteria were: age $\leq$40 years, body mass index (BMI) $<$26 kg/m^2, and singletons born alive after the 20th gestational week. A maximum of two embryos were allowed to be transferred. Women who delivered singletons after more than one gestational sac was seen on the first-trimester ultrasound, namely vanishing twins, were excluded. In the case of patients who had more than one delivery during the study period, only the first pregnancies were included for analysis. Furthermore, considering pregnancy-related factors possibly affecting intrauterine fetal growth, patients with gestational diabetes, pregnancy-induced hypertension, and preeclampsia were also excluded. This study was approved by the Institutional Review Board of the hospital. All participants gave informed consent.

Laboratory Protocols

Details on ovarian stimulation, oocyte retrieval, and IVF/intracytoplasmic sperm injection (ICSI) procedures have been previously described (18, 19). Briefly, conventional IVF or ICSI was performed based on semen parameters and previous fertilization history. For IVF, oocytes were inseminated with human tubal fluid (HTF; Irvine Scientific) supplemented with 10% serum substitute supplement (SSS; Irvine Scientific) and ~300 000 progressively motile spermatozoa. In case of ICSI, oocytes were transferred to fertilization medium (HTF + 10% SSS) immediately after microinjection. Fertilization was checked 16–18 hours after insemination/microinjection. Then zygotes were transferred into a dish containing preequilibrated continuous single culture medium (CSC; Irvine Scientific) plus 10% SSS for individualized culture, without medium renewal up to the day of embryo frozen.

All embryos were incubated under oil at 37°C in a 5% O_2 and 6% CO_2 environment. Embryo morphology assessment was performed on day 3, day 5, and day 6. Type of culture medium and laboratory protocols and conditions remained constant during the study period.

Endometrial Preparation Before Embryo Transfer and Vitrification

Modified natural cycles were applied for patients with regular ovulatory cycles, with the use of hCG for scheduling embryo transfer. In patients with irregular menses, an artificial cycle was offered with sequential administration of estrogen and progesterone. The timing of transfer was planned depending on the embryonic developmental stage on the day of vitrification. The number of embryos transferred was based on patient age as well as previous IVF cycles, and a maximum of two embryos were allowed to be transferred.

The vitrification and thawing procedure was previously described by Kuwayama et al. (20). Briefly, embryo vitrification was carried out via Cryotop carrier system, in conjunction with dimethylsulfoxide–ethylene glycol–sucrose as cryoprotectants. For thawing, embryos were transferred into dilution solution in a sequential manner (1 mol/L to 0.5 mol/L to 0 mol/L sucrose).

Outcome Measures and Definitions

In FET cycles, gestational age was calculated from the day of embryo transfer, which was defined as day 17 of the cycle for cleavage-stage embryo transfer and day 19 for blastocyst transfer (21). Low birth weight (LBW), very LBW, and high birth weight (HBW) were defined as birth weights $<$2,500 g, $<$1,500 g, and $>$4,500 g, respectively. Preterm birth and very preterm birth were defined as delivery before 37 and 32 completed gestational weeks, respectively.

Small for gestational age (SGA) and very SGA were defined as birth weights $<$10th and $<$3rd percentiles, respectively. Large for gestational age (LGA) and very LGA were defined as birth weights $>$90th and $>$97th percentiles, respectively. In addition, *Z*-score was adopted to calculate birth weight adjusted for neonatal sex and gestational age according to the following equation: $Z\text{-score} = (x - \mu)/\sigma$, in which x is the weight of an infant, μ is the mean birth weight for the same sex and same gestational age in the reference group, and

σ is the standard deviation of the reference group. Birth weight percentiles and calculation of *Z*-scores were based on Chinese reference singleton newborns stratified by gestational age and neonatal sex (22). All neonatal and delivery information were obtained from electronic medical records.

Statistical Analysis

The baseline characteristics and neonatal outcomes were compared between the study groups via either *t* test (for continuous variables) or χ^2 test (for categoric variables). To investigate the effect of extended culture on adverse perinatal outcomes while controlling for other potential confounders, a multivariable regression model was introduced.

A multiple linear regression analysis was performed to survey the relationship between embryo culture duration and neonatal birth weight with adjustment for possible confounding factors, including maternal age, maternal BMI, paternal age, paternal BMI, parity, infertility cause, infertility duration, fertilization method (standard IVF or ICSI), FET cycle rank, sperm origin (testicular sperm extraction or ejaculation), endometrial preparation protocol (modified natural cycle or artificial cycle), number of embryos transferred, newborn sex, and gestational age at birth. All statistical analyses were performed with the use of the Statistical Package for Social Sciences (SPSS) version 21.0. A *P* value of $<.05$ was considered to be statistically significant.

RESULTS

A total of 4,201 women fulfilling the study inclusion criteria were enrolled and gave birth to 3,520, 215, and 466 live-born singletons after, respectively, day 3, day 5, and day 6 vitrified-warmed embryo transfer. The main maternal and treatment characteristic of included cycles are presented in Table 1. No difference was observed in maternal age and BMI, infertility cause, maternal smoking behavior, obstetrical history (parity and gravidity), infertility duration, and method of endometrial preparation between the two groups. Nonetheless, a significant difference existed in distribution of FET rank and number of embryos transferred. Furthermore, ICSI was more frequently performed in day 3 transfers. These parameters would be entered as potential confounders into a linear regression analysis.

The neonatal outcomes according to culture duration are presented in Table 2. Sex ratio was skewed, with more males born after day 6 compared with day 3 transfers (57.1% vs.

TABLE 1

Maternal and treatment characteristics of the vitrified-thawed cycles.

Characteristic	Vitrified day 3 (n = 3,520)	Vitrified day 5 (n = 215)	Vitrified day 6 (n = 466)	*P* value[a]	*P* value[b]
Maternal age (y)	31.90 ± 3.86	31.49 ± 3.73	31.82 ± 3.77	.130	.679
Maternal BMI (kg/m^2)	20.85 ± 2.19	20.89 ± 2.13	20.68 ± 2.11	.771	.126
Paternal age (y)	33.95 ± 4.99	33.60 ± 4.90	33.56 ± 4.96	.310	.116
Paternal BMI (kg/m^2)	23.68 ± 2.71	23.78 ± 2.84	23.45 ± 2.72	.294	.086
Infertility cause				.112	.102
Female	2,092 (59.4%)	135 (62.8%)	269 (57.7%)		
Male	370 (10.5%)	30 (14.0%)	36 (7.7%)		
Mixed	880 (25.0%)	42 (19.5%)	132 (28.3%)		
Unexplained	178 (5.0%)	8 (3.7%)	29 (6.2%)		
Parity				.750	.736
First	3,278 (93.1%)	199 (92.6%)	432 (92.7%)		
High order	242 (6.9%)	16 (7.4%)	34 (7.3%)		
Maternal Smoking	40 (1.1%)	2 (0.9%)	6 (1.3%)	.781	.774
Infertility duration (y)	3.38 ± 2.69	3.27 ± 2.54	3.44 ± 2.56	.581	.662
FET cycle rank				<.001	<.001
First	2,154 (61.2%)	69 (32.1%)	144 (30.9%)		
High order	1,366 (38.8%)	146 (67.9%)	322 (69.1%)		
Sperm origin				.938	.666
Ejaculation	3,441 (97.8%)	210 (97.7%)	457 (98.1%)		
Testicular sperm extraction	79 (2.2%)	5 (2.3%)	9 (1.9%)		
Fertilization method				.037	.015
IVF	2,272 (64.5%)	141 (65.6%)	326 (70.0%)		
ICSI	929 (26.4%)	45 (20.9%)	94 (20.2%)		
IVF + ICSI	319 (9.1%)	29 (13.5%)	46 (9.9%)		
FET endometrial preparation				.121	.169
Modified natural cycle	1,698 (48.2%)	92 (42.8%)	209 (44.8%)		
Artificial cycle	1,822 (51.8%)	123 (57.2%)	257 (55.2%)		
No. of embryos transferred				<.001	<.001
1	397 (11.3%)	136 (63.3%)	269 (57.7%)		
2	3,123 (88.7%)	79 (36.7%)	197 (42.3%)		

Note: Data are presented as mean ± SD for continuous variables and n (%) for dichotomous variables. All *P* values were assessed with the use of χ^2 or Student *t* tests. BMI = body mass index; FET = frozen-thawed embryo transfer; ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization.

[a] Day 5 vs. day 3.

[b] Day 6 vs. day 3.

Zhang. Higher birth weight after blastocyst FET. Fertil Steril 2018.

TABLE 2

Neonatal outcomes of live-born singletons according to culture duration.

Outcome	Vitrified day 3 (n = 3,520)	Vitrified day 5 (n = 215)	Vitrified Day 6 (n = 466)	*P* value[a]	*P* value[b]
Newborn sex				.096	.020
Female	1,712 (48.6%)	92 (42.8%)	200 (42.9%)		
Male	1,808 (51.4%)	123 (57.2%)	266 (57.1%)		
Gestational age (wk)				.168	.228
<32	16 (0.5%)	3 (1.4%)	1 (0.2%)		
32–36	142 (4.0%)	9 (4.2%)	26 (5.6%)		
≥37	3,362 (95.5%)	203 (94.4%)	439 (94.2%)		
Birth weight (g)	3,377.65 ± 456.98	3,417.51 ± 505.47	3,412.87 ± 481.47	.217	.130
Z-score	0.38 ± 1.01	0.57 ± 1.08	0.50 ± 1.04	.011	.026
Low birth weight (<2,500 g)	102 (2.9%)	6 (2.8%)	13 (2.8%)	.928	.900
Very low birth weight (<1,500 g)	6 (0.2%)	1 (0.5%)	1 (0.2%)	.332	.831
High birth weight (>4,500 g)	26 (0.7%)	3 (1.4%)	2 (0.4%)	.287	.452
Small for gestational age (<10th percentile)	147 (4.2%)	8 (3.7%)	18 (3.9%)	.745	.750
Large for gestational age (>90th percentile)	626 (17.8%)	55 (25.6%)	110 (23.6%)	.004	.002
Very small for gestational age (<3rd percentile)	47 (1.3%)	2 (0.9%)	5 (1.1%)	.612	.639
Very large for gestational age (>97th percentile)	224 (6.4%)	21 (9.8%)	45 (9.7%)	.050	.008

Note: Data are presented as mean ± SD for continuous variables and n (%) for dichotomous variables. *Z*-scores are adjusted for sex and gestational age. All *P* values were assessed with the use of χ^2 or Student *t* tests.
[a] Day 5 vs. day 3.
[b] Day 6 vs. day 3.

Zhang. Higher birth weight after blastocyst FET. Fertil Steril 2018.

51.4%; *P*=.02). The mean birth weights were 3,377.65 ± 456.98 g, 3,417.51 ± 505.47 g, and 3,412.87 ± 481.47 g in the day 3, day 5, and day 6 groups, respectively, not reaching statistical significance (day 3 vs. day 5: *P*=.217; day 3 vs. day 6: *P*=.13). However, a higher *Z*-score was observed in the day 5 (0.57 ± 1.08; *P*=.011) and day 6 (0.50 ± 1.04; *P*=.026) groups than in the day 3 group (0.38 ± 1.01).

As presented in Table 3, the rate of LGA babies was higher in the day 5 and day 6 groups compared with the day 3 group after adjusting for known confounding factors (adjusted odds ratio [aOR] 1.64, 95% confidence interval [CI] 1.16–2.33, and aOR 1.24, 95% CI 1.09–1.41, respectively). Also, the odds of very LGA singletons was significantly higher in the day 6 group compared with the day 3 group (aOR 1.35, 95% CI 1.12–1.64). The other neonatal parameters, including the incidences of LBW, very LBW, HBW, preterm birth, SGA, and very SGA, were similar between groups.

Multiple linear regression analyses were performed to determine the relationship between the duration of in vitro culture and singleton birth weight (Table 4). Even after correction for several potential confounders, a positive association was found in a multiple linear regression model between birth weight and culture duration: day 5 vs. day 3: β = 90.1 g (SE 29.24 g; *P*=.002); day 6 vs. day 3: β = 58.73 g (SE 21.26 g; *P*=.006). Moreover, maternal BMI (*P*<.001), paternal BMI (*P*=.004), newborn sex *P*<.001), and gestational age at birth (*P*<.001) were all independent predictors of birth weight.

DISCUSSION

To the best of our knowledge, our study is the first one solely focusing on the effect of embryo culture period on singleton birth weight after vitrified-warmed transfer cycles. In line with most previous observation studies based on fresh cycles,

TABLE 3

The risk of being born LGA and very LGA in singletons after cleavage-stage (day 3) versus blastocyst (day 5 and day 6) transfer.

	Day 5 vs. day 3		Day 6 vs. day 3	
Outcome	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Very preterm (<32 wk)	3.10 (0.90–10.72)	2.66 (0.64–11.01)	0.47 (0.06–3.56)	0.67 (0.23–1.95)
Preterm (<37 wk)	1.26 (0.69–2.30)	1.13 (0.59–2.17)	1.31 (0.86–1.99)	1.08 (0.85–1.37)
Low birth weight (<2,500 g)	0.96 (0.42–2.22)	0.91 (0.37–2.22)	0.96 (0.54–1.73)	0.98 (0.71–1.35)
Very low birth weight (<1,500 g)	2.73 (0.33–22.83)	1.65 (0.16–17.62)	1.26 (0.15–10.49)	1.19 (0.37–3.78)
High birth weight (>4,500 g)	1.90 (0.57–6.33)	2.17 (0.58–8.17)	0.58 (0.14–2.45)	0.78 (0.36–1.69)
Small for gestational age (<10th percentile)	0.89 (0.43–1.83)	0.68 (0.31–1.46)	0.92 (0.56–1.52)	0.87 (0.66–1.14)
Large for gestational age (>90th percentile)	1.59 (1.16–2.19)	1.64 (1.16–2.33)	1.43 (1.13–1.80)	1.24 (1.09–1.41)
Very small for gestational age (<3rd percentile)	0.69 (0.17–2.88)	0.59 (0.13–2.63)	0.80 (0.32–2.03)	0.91 (0.55–1.50)
Very large for gestational age (>97th percentile)	1.60 (1.00–2.55)	1.66 (0.98–2.80)	1.57 (1.12–2.20)	1.35 (1.12–1.64)

Note: Analyses were adjusted for maternal age, maternal body mass index, smoking, parity, newborn sex, frozen-thawed embryo transfer (FET) cycle rank, fertilization method, FET endometrial preparation, and number of embryos transferred. CI = confidence interval; OR = odds ratio.

Zhang. Higher birth weight after blastocyst FET. Fertil Steril 2018.

TABLE 4

Results of multiple regression analysis of birth weight among live born singletons following vitrified-thawed transfer.

	Unstandardized coefficients		Standardized coefficients		
Model	**B**	**Std. Error**	**Beta**	***t***	***P* value**
(Constant)	−4,110.760	217.242		−18.922	<.001
Day 5 (vs. day 3)	90.101	29.243	0.043	3.081	.002
Day 6 (vs. day 3)	58.729	21.257	0.040	2.763	.006
Maternal age (y)	−2.476	2.344	−0.021	−1.056	.291
Maternal BMI (kg/m^2)	24.660	2.817	0.116	8.754	<.001
Maternal smoking	94.897	57.108	0.022	1.662	.097
Parity: high order (vs. first)	19.356	24.571	0.011	0.788	.431
Paternal age (y)	3.331	1.752	0.036	1.901	.057
Paternal BMI (kg/m^2)	6.534	2.242	0.038	2.914	.004
Infertility cause					
Female (reference)					
Male	21.419	24.284	0.014	0.882	.378
Mixed	−18.901	15.768	−0.018	−1.199	.231
Unexplained	7.961	29.050	0.004	0.274	.784
Infertility duration (y)	−0.698	2.386	−0.004	−0.293	.770
FET cycle rank: high order (vs. first)	15.517	12.666	0.017	1.225	.221
Fertilization method					
IVF (reference)					
ICSI	−19.743	17.064	−0.019	−1.157	.247
IVF + ICSI	2.182	22.319	0.001	0.098	.922
FET endometrial preparation: artificial cycle (vs. modified natural cycle)	4.048	6.108	0.009	0.663	.507
No. of embryos transferred	28.515	17.315	0.024	1.647	.100
Gestational age (wk)	166.992	4.332	0.510	38.551	<.001
Newborn sex: male (vs. female)	140.904	12.183	0.152	11.565	<.001
Sperm origin, TESE (vs. ejaculation)	84.590	44.551	0.027	1.899	.058

Note: TESE = testicular sperm extraction; other abbreviations as in Table 1.

Zhang. Higher birth weight after blastocyst FET. Fertil Steril 2018.

the present study showed that *Z*-scores were significantly higher in blastocyst transfer than embryo culture limited to 3 days, although no significant difference existed in mean birth weight between the study groups. Likewise, the proportion of LGA babies was higher in the blastocyst group than in the day 3 group.

Over the past decade, whether in vitro embryo culture period affects neonatal birth weight has been a subject of much debate (10–15). Possible factors accounting for the discrepancies between studies are many. First, the majority of the studies suffer from small sample size, preventing them from drawing firm conclusions. In addition, existing studies are heterogeneous, including methodology, study population and potential confounders, all of which may have compromised the results. Furthermore, different types of commercial culture media used in IVF treatment may partially explain conflicting results.

Notably, most of the aforementioned studies mainly focused on fresh embryo transfer cycles, without ruling out the possibility of adverse fetal growth caused by a hyperestrogenic milieu. A number of investigators have indicated that supraphysiologic E_2 levels produced by controlled ovarian stimulation may create a suboptimal periimplantation environment, leading to placental dysfunction and, therefore, LBW (16, 23). This adverse perinatal outcome can be mitigated by frozen-thawed embryo replacement and natural IVF cycles (24, 25). In the present study exclusively based on FET cycles, we eliminated hyperestradiol-mediated effects on fetal growth to determine the exact role of culture duration in subsequent birth weight. Our findings further suggested that prolonged in vitro culture had a significant impact on singleton birth weight and that this impact can not be overcome by transfer of embryos into a more physical uterine environment.

In contrast to our findings, a very recent study by de Vos et al. reported a significantly lower birth weight *Z*-score in singletons born after vitrified-warmed blastocyst transfer compared with cleavage-stage transfer (17). These contradictory findings could be largely attributed to the limited population size included in the de Vos et al. study (a total of 116 singletons in vitrified-warmed transfer cycles: 58 in the day 3 group and 58 in the day 5 group). Furthermore, possible factors known to affect intrauterine fetal growth, such as pregnancy-induced hypertension, gestational diabetes, and preeclampsia were excluded from our study, which de Vos et al. failed to do. Finally, different commercial culture media were used in the two studies (Quinn Advantage protein plus sequential media in De Vos et al. and continuous single culture medium in the present study). It has been suggested that culture media compositions have potential influence on newborn birth weight (26).

The exact mechanism of the association between newborn birth weight and culture duration remains unknown. Currently available data on this topic mostly come from nonhuman animal research. As early as 1998, Young et al. proposed that exposure of bovine and ovine embryos

to in vitro culture resulted in large offspring syndrome (27). Further evidence has provided new insight that the early embryonic development stage is vulnerable and sensitive to its environmental conditions, such as in vitro culture, and that any perturbation in this process would force embryos to make adaptations via epigenetic alternations, leading to alterations in fetal growth trajectory and therefore birth weight (28, 29). Numerous recently published studies have identified that the composition of culture medium could interfere with gene expressions and DNA methylation patterns in mouse preimplantation embryos compared with in vivo counterparts (30, 31). Interestingly, these effects can be maintained, or even be more pronounced, after freezing and thawing of the embryos (4). Nevertheless, the above findings obtained from nonhuman studies can not be extrapolated to patients undergoing assisted reproduction. Future research shedding new light on this issue in humans is urgently needed.

Multiple linear regression analysis was carried out to assess the relationship between possible confounders and singleton birth weight. Maternal BMI, paternal BMI, gestational age at birth, and neonatal sex were all significantly correlated to birth weight, which was well in line with previous literatures. High parity has been reported to be closely related to the newborns' birth weight, which was not seen in the present study. This difference may be due to the fact that the vast majority of patients included were nulliparous, such that power was lacking to detect such an effect, or it may be due to the freeze-thaw process itself (32).

In our cohort, the odds of LGA singletons was significantly higher with blastocyst transfer. This finding had been described by other investigators (10, 11). Nonetheless, the incidence of LGA was higher than reported in previous studies based on vitrified-warmed cycles (33–35), probably because the definition of LGA was more strict in those studies (defined as Z-score >2). When applying the same definition, our reported data well concurred with a large Japanese study on perinatal outcomes after single-embryo transfer of vitrified embryos (36). Unfortunately, the Japanese study failed to separately present data on the basis of embryonic stage. Thereafter, whether extended in vitro culture plus the freeze-thaw process would further exacerbate the incidence of LGA still awaits further confirmation.

The addition of proteins to culture media for human embryo culture is common. The choice of protein supplement and concentration varies greatly among IVF laboratories. Presently, the most frequently used protein source is human serum albumin (HSA). Compared with HSA, SSS contains 16% α- and β-globulins in addition to HSA. A large randomized controlled study showed adding SSS to commercial HSA-supplemented embryo culture media resulted in higher implantation and live birth rates compared with HSA as the sole protein supplement (37). However, without sufficient evidence, a solid conclusion can not be drawn regarding the superiority of any one protein support over another for human embryo culture. Furthermore, it is still unknown to what extent heterogeneity in different preparations of HSA affects the development of human embryos in vitro and perinatal outcomes such as birth weight (9).

The present study is limited by the bias inherent in its retrospective nature. In this regard, we meticulously screened the database with strict criteria. The strength of the study is that we were able to eliminate possible confounders related to newborn birth weight, including vanishing twins and pregnancy complications; not all of the existing studies evaluating newborn birth weight have consistently done so. In addition to introducing a linear regression model to control for possible confounders, Z-score was calculated to further clarify the effect on birth weight of the time embryos spent in culture. Most importantly, in the present study, laboratory conditions and procedures as well as culture medium remained consistent during the study period.

CONCLUSION AND FUTURE PROSPECTS

In summary, this single-center retrospective study showed that both the sex- and gestational age–adjusted birth weight (Z-score) and the proportion of LGA infants were significantly higher in blastocyst than in cleavage-stage transfer after vitrified-warmed cycles. This finding should be confirmed by future large prospective studies.

The ultimate goal of IVF specialists is not only to help couples establish a successful pregnancy, but also to help them deliver a healthy normal-weight baby at full term, in the long term resulting in a carefree childhood and subsequently a healthy adult. It is too early to know if the difference in birth weight that we found in the present study has any clinical significance. Therefore, ongoing follow-up studies are under investigation in our center to determine whether extended in vitro culture has lasting consequences on IVF offspring.

Acknowledgments: The authors thank the nurses and laboratory staff of the Department of Assisted Reproduction for their contribution to this work. Moreover, the authors thank the infertile couples who participated in this study.

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Efecto del cultivo *in vitro* en el peso al nacer después de los ciclos con transferencia de embrión vitrificado-descongelado: análisis de 4.201 recién nacidos

Objetivo: Investigar el efecto del cultivo de embriones en el peso al nacer tras los ciclos de vitrificado-descongelado.

Diseño: Estudio de cohortes retrospectivo.

Lugar: Centre médico académico de atención terciaria.

Paciente(s): Un total de 4.201 mujeres que dieron a luz a 3.520, 215 y 466 recién nacidos vivos después de la transferencia de blastocistos en etapa de escisión congelados-descongelados en día 3, día 5 y día 6, respectivamente.

Intervenciones: Ninguna

Mediadas de los resultados principales: Peso neonatal

Resultado(s): El peso medio al nacer no difirió entre los tres grupos de estudio. Sin embargo, el peso al nacer ajustado a la edad gestacional y el sexo (Z-scores) y la proporción de bebés de talla grande en relación a la edad gestacional (GEG) fue significativamente mayor después de transferir embriones en día 5 y día 6 que después de la transferencia de embriones en día 3. Además, el análisis de regresión lineal múltiple indicó que la edad gestacional, el índice de masa corporal de la madre, el sexo del neonato y la duración del período de cultivo, tuvieron un significativo y fuerte impacto sobre el peso al nacer de los recién nacidos.

Conclusión(es): En los ciclos con transferencia de embriones tras vitrificado-descongelado, los Z-scores del peso al nacer y la proporción de bebes de talla grande (GEG) fue mayor en recién nacidos después de la transferencia de blastocistos, que después de la transferencia de embriones en etapa de escisión. Este hallazgo sugiere que el efecto de la duración del cultivo supera el beneficio de la transferencia de embriones en condiciones más parecidas al contexto fisiológico uterino.