

# The effect of post-thaw culture duration on singleton birthweight resulting from frozen embryo transfer cycles

Hongliang Yi, Chengguang Sun, Zhi Yuan, Huijia Chen, Chanyu Zhang\*

The Reproductive Medical Center, The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China

**Submitted:** 9 May 2023; **Accepted:** 6 November 2023

**Online publication:** 19 August 2024

Arch Med Sci 2025; 21 (3): 852–857  
DOI: <https://doi.org/10.5114/aoms/174868>  
Copyright © 2024 Termedia & Banach

**\*Corresponding author:**

Chanyu Zhang, MD  
The Reproductive  
Medical Center of  
The Second Affiliated  
Hospital of Chongqing  
Medical University  
Fax: +86(023)-62888342  
E-mail: 1317954623@qq.com

## Abstract

**Introduction:** We aimed to explore the possible link between the post-thaw culture duration and singleton birthweight resulting from frozen embryo transfer (FET) cycles.

**Material and methods:** In this retrospective study, 216 singletons were analyzed between January 2017 and December 2018 in our center. FET cycles were allocated to two main groups based on the interval between the moment of thawing and embryo transfer. The main outcome measure was the birthweight of singletons after FET. The confounding factors for singleton birthweight were evaluated using multiple linear regression analysis.

**Results:** The mean singleton birthweight resulting from the long culture transfer group was not significantly different from singletons resulting from the short culture transfer group. The z-scores for singletons were also not significantly different; however, the long culture transfer group had a higher proportion of small for gestational age (SGA) than the short culture transfer group. Using multiple linear regression analysis, we found that infant gender, gestational age and maternal BMI significantly affect singleton birthweight, while the duration of post-thaw culture had no effects on newborn birthweight.

**Conclusions:** Our study suggested that post-thaw culture duration did not affect the birthweight of singleton newborns. But singleton newborns from long post-thaw culture after a hormone replacement therapy (HRT) may have a higher risk of SGA.

**Key words:** frozen embryo transfer, culture duration, birthweight, small for gestational age.

## Introduction

The advancement of vitrification methods and the broad acceptance of single embryo transfer have contributed to the global increase in the usage of frozen embryo transfer (FET) [1, 2]. After warming, embryos usually need to be cultured *in vitro* to help embryo recover and verify their viability. Usually, two basic options are available according to the FET strategy: transfer into the uterus after a short post-thaw culture duration and transfer after overnight culture. There is conflicting evidence concerning the effect of the duration of post-thaw culture on reproductive outcomes, an overall consensus regarding the optimal time interval between thawing and embryo transfer does not yet exist. Several studies have shown

that a short post-thaw culture period led to higher rates of pregnancy and live birth than overnight culture [3–5]. Some studies, however, found no differences in reproductive results between the short and long culture groups [6–8]. Recently, Zhu *et al.* observed the impact of different post-thaw culture periods on reproductive outcomes after FET of cleavage-stage embryos and their results indicated that the clinical pregnancy outcomes were highly correlated with the duration of post-thaw culture [9]. Nonetheless, the studies mentioned above mostly focused on comparing pregnancy rates, implantation rates and live birth rates between the short and long post-thaw culture duration. Little attention has been paid to address the impact of post-thaw culture period on singleton birthweight. Therefore, the present study aimed to determine whether the different durations of post-thaw cultures of thawed cleavage-stage embryos significantly influenced the birthweight of singletons derived from FET cycles.

## Material and methods

### Patients and study groups

We analyzed retrospectively singleton babies born through the first vitrified-warmed cleavage-stage embryo transfer cases performed in our clinical center between January 2017 and December 2018. According to the *in vitro* culture duration between thawing and transfer, the patients were assigned into the short culture group (2 h) and the long culture group (overnight culture, 20 h). Data from singleton deliveries occurring at a gestational age of more than 20 weeks were included. The infants from donor oocytes, donor sperm, vanishing twins and pre-implantation genetic testing (PGT) were all excluded from this study.

### Cryopreservation and warming protocols

The vitrification and thawing method was previously described by Kuwayama [10]. During the complete study period, the vitrification and thawing procedures and medium remained unchanged. After thawing, embryos were checked for morphological blastomere survival immediately. Only embryos that exhibited more than 50% intact blastomeres were considered viable and suitable for transfer. An embryo with 7 to 9 equal cells and < 10% fragmentation was classified as good quality.

### FET and follow-up

There are two main clinical protocols for preparing endometrium. Natural cycles (NC) are the preferred method for patients whose ovulatory cycles are regular. The hormone replacement therapy (HRT) cycle was considered a favorable choice

for patients with a history of irregular menstruation or anovulation. All details about endometrial preparation were described previously [11]. Two good quality embryos in cleavage stage were transferred into the uterus when the endometrium reached adequate thickness. 28 days after FET, the presence of a gestational sac and fetal heartbeat diagnosed by ultrasound was defined as clinical pregnancy.

### Outcome measures

Birth information of singleton newborns, including gestational age and weight, were obtained from obstetric records. Birthweight was the main outcome measure in our analysis. Other evaluated neonatal outcome parameters included very low birthweight, low birthweight, high birthweight, very preterm birth, preterm birth, small for gestational age (SGA), and large for gestational age (LGA) were in accordance with the published literature [12]. In addition, the z-scores were adopted to estimate gestational age- and sex-adjusted birthweight, as previously described [13]. The reference population and calculation of z-scores, SGA, and LGA were all based on a study involving singleton birthweight of Chinese newborns [14].

### Statistical analysis

The clinical characteristics, obstetrical and neonatal outcomes were compared using Student's *t*-test (for continuous variables) or the  $\chi^2$  test (for categorical variables) between the study groups. A multiple linear regression analysis was performed to examine the possible associations between birthweight and post-thaw culture duration with other maternal potential confounding factors (maternal age, maternal BMI, type of infertility, infertility cause, parity, duration of infertility, gestational age, infant gender). All statistical analyses were conducted using SPSS 21.0 and the level of significance was 5%.

## Results

### Maternal and treatment characteristics based on post-thaw culture duration

In the present study, a total of 216 singletons resulting from frozen-thawed embryo transfer cycles were included. The treatment and main maternal characteristics according to the post-thaw culture duration are presented in Table I. No differences in maternal age, maternal body mass index (BMI), maternal parity, infertility cause, and duration of infertility were observed between the two groups, except for endometrial preparation. These parameters were incorporated into a linear regression analysis as potential confounders.

**Table I.** Maternal clinical and treatment characteristics

Variable	Short culture group	Long culture group	P-value
Cycles/singletons (n)	120	96	–
Maternal age [years]	32.2 ±3.6	32.4 ±4.2	0.772
Maternal BMI [kg/m <sup>2</sup> ]	21.3 ±2.6	21.1 ±2.2	0.393
Maternal parity:			
First	114 (95.0%)	91 (94.8%)	0.945
High order	6 (5.0%)	5 (5.2%)	
Maternal smoking:			
Non-smoker	118 (98.3%)	93 (96.9%)	0.479
Smoker	2 (1.7%)	3 (3.1%)	
Main infertility cause:			
Female	93 (77.5%)	70 (72.9%)	0.874
Male	8 (6.7%)	8 (8.3%)	
Mixed	14 (11.6%)	14 (14.6%)	
Unexplained	5 (4.2%)	4 (4.2%)	
Type of infertility:			
Primary	58 (48.3%)	48 (50.0%)	0.808
Secondary	62 (51.7%)	48 (50.0%)	
Duration of infertility [years]	4.2 ±2.8	4.0 ±3.0	0.454
Endometrial preparation:			
Natural cycle	37 (30.8%)	9 (9.4%)	0.001
HRT cycle	83 (69.2%)	87 (90.6%)	

Data are presented as numbers (%) or the mean ± SD. Continuous variables were compared using analysis of variance, and the  $\chi^2$  test compared categorical variables.

### Perinatal outcome according to post-thaw culture duration

Table II shows the perinatal parameters of the singletons. No differences in mean singleton birthweight were observed between the two groups. Moreover, there was no significant difference in the gestational age and infant gender distribution between the two study groups. We calculated the gestational age- and sex-adjusted birthweight (z-scores) of all neonates to further investigate the impact of post-thaw culture duration on birthweight. No statistically significant difference in z-scores was observed between the two groups. However, a higher proportion of SGA was observed in the long culture transfer group than in the short culture transfer group.

### Multiple linear regression analysis

To determine the associations between post-thaw culture period or other parameters and birthweight of the live born singletons, we also carried out multiple linear regression analysis. As shown in Table III, gestational age, infant gender and maternal BMI significantly affected the birthweight of the live born singletons. However, no positive relationship was demonstrated in a mul-

tiple linear regression model between singleton birthweight and post-thaw culture duration.

### Discussion

The present study was designed to investigate the impact of post-thaw culture duration on singleton birthweight following FET cycles. Overall, our study shows that different *in vitro* culture durations from warming to transfer of cleavage-stage embryos have similar birthweight. Likewise, singleton newborns from long post-thaw culture after a hormone replacement therapy (HRT) may have a higher risk of SGA.

A major focus among couples undergoing infertility treatment is the long-term safety of newborns. Numerous studies have established that babies from FET cycles appear to have similar perinatal outcomes when compared to babies born after fresh transfer cycles [15]. Nevertheless, when compared to children who were conceived naturally, infants conceived via FET are more likely to have a lower birthweight [16]. Because an abnormal birthweight is associated with significant adverse effects on health outcomes during the fetal and neonatal periods [17], improving infant birthweights conceived through ART is crucial to improving overall ART outcomes.

**Table II.** Infant clinical characteristics

Variable	Short culture group	Long culture group	P-value
Gestational age [weeks]:	39.1 ±1.3	39.1 ±1.4	0.344
Very preterm, < 32 weeks	0 (0.0%)	0 (0.0%)	
Preterm, 32–36 weeks	5(4.2%)	5 (5.2%)	
Normal, ≥ 37 weeks	115 (95.8%)	91 (94.8%)	
Newborn gender:			0.067
Female	70 (58.3%)	44 (45.8%)	
Male	50 (41.7%)	52 (54.2%)	
Mean birthweight [g]:	3428 ±410.9	333 ±484.8	0.131
Normal weight, 2500–4000 g	112 (93.3%)	84 (87.6%)	
Very low birthweight, < 1500 g	0 (0.0%)	0 (0.0%)	
Low birthweight, 1500–2500 g	1 (0.8%)	6 (6.2%)	
High birthweight, > 4000 g	6 (5.1%)	5 (5.2%)	
Very high birthweight, > 4500 g	1 (0.8%)	1 (1.0%)	
Small for gestational age (< 10 <sup>th</sup> percentile)	2 (1.7%)	10 (10.4%)	0.001
Large for gestational age (> 90 <sup>th</sup> percentile)	20 (16.7%)	14 (14.6%)	0.624
Mean birthweight z-score	0.479	0.282	0.157
95% CI	0.313 to 0.650	0.052 to 0.496	

Data are presented as numbers (%) or the mean ± SD. Continuous variables (gestational age and birthweight) were compared by Student's *t*-test, and the  $\chi^2$  test compared categorical variables (newborn gender).

**Table III.** 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	-2665.226	924.696		-2.882	0.004
Culture duration	-70.722	57.853	-0.079	-1.222	0.223
Maternal age [years]	-4.319	7.918	-0.037	-0.545	0.586
Maternal BMI [kg/m <sup>2</sup> ]	27.704	11.355	0.152	2.440	0.016
Maternal smoking	179.811	183.490	0.061	0.980	0.328
Maternal parity	170.128	131.496	0.084	1.294	0.197
Type of infertility	100.149	58.905	0.112	1.700	0.091
Main infertility cause	-3.526	32.665	-0.007	-0.108	0.194
Duration of infertility [years]	6.051	10.537	0.039	0.574	0.566
Endometrial preparation	6.298	69.712	0.006	0.090	0.928
Gestational age [weeks]	137.030	21.153	0.406	6.478	0.001
Newborn gender	-134.105	56.288	-0.150	-2.382	0.018

It is generally accepted that several factors, including maternal factors, ovarian stimulation, culture medium, and cryopreservation, are associated with adverse neonatal outcomes [18]. Another factor, the duration of post-thaw culture may also have an important influence on clinical outcomes, including birthweight [19].

Over the last decade, researchers have argued whether *in vitro* embryo culture duration affects newborn birthweight [20]. Prior studies mainly focused on the clinical outcomes of fresh embryo transfer cycles. Exogenous gonadotrophin stimulation has been demonstrated to influence

the maternal uterine environment, resulting in fetal growth restriction and low birthweight [21]. To avoid the possible adverse effects of supraphysiologic E<sub>2</sub> on the birthweight of the live born singletons, FET cycles were analyzed in the present study to investigate the role of post-thaw culture duration in subsequent birthweight. Our retrospective study found that the duration of post-thaw culture did not significantly influence the mean birthweight and z-scores in live born singletons in FET cycles. In contrast to our findings, Zhang *et al.* found that the culture duration had a strong and significant effect on newborn

birthweight. The birthweight z-scores increase with a longer *in vitro* culture duration [22]. However, a recent study by de Vos *et al.* found that singletons born following vitrified-warmed blastocyst transfer had significantly lower birthweight z-scores than cleavage-stage embryo transfer [23]. As we know, during an overnight culture, environmental conditions may significantly influence embryo implantation and developmental potential. Even though the difference in post-warming culture time was nearly 24 h, it was insufficiently long to have an impact on singleton birthweight. Furthermore, Zhu *et al.* demonstrated that even though frozen-thawed cleavage-stage embryos were cultured to the blastocyst stage, no correlation was observed between the duration of culture and birthweight of singleton newborns [9]. There are numerous factors that may account for these contradictory findings. First, the majority of the studies were limited by a small sample size of live birth (a total of 116 and 216 singletons included in the de Vos *et al.* and our study, respectively). In addition, the study method and population were different, all of which may have affected the study results. Furthermore, different types of culture medium were used for embryo culture (continuous single culture medium in Zhang *et al.* and Quinn Advantage sequential media in the present study) and several researches have confirmed previous concerns that the type of culture media may have an impact on singleton birthweight [24–26].

The effect of *in vitro* culture duration on the odds of LGA singletons is still a matter of debate. Zhang *et al.* reported that the odds of LGA births was significantly higher after blastocyst transfer [22]. However, another study revealed that *in vitro* culture duration had no effect on the proportion of LGA births from FET cycles [27]. Surprisingly, our retrospective clinical study showed that long post-thaw culture period was associated with a higher proportion of SGA infants from FET cycles. One of important possible factors that account for these contradictory findings was the protocol used to prepare the endometrium. The endometrial preparation protocol has been found to be an independent factor associated with later fetal growth following FET [28]. Although FET could effectively prevent patients from suffering the negative consequences of superphysiological hormonal levels during the first trimester, estrogen supplementation for HRT would still have some effect on maternal blood estradiol levels. Impaired uteroplacental blood flow may have an impact on the growth and development of fetal and neonatal outcomes, such as SGA [29]. A study by Li *et al.* indicated that HRT protocols increased the risk of SGA of singletons born after FET [30]. It is therefore not unexpected to find that the pro-

portion of SGA was higher in the long post-thaw culture group.

Multiple linear regression analysis is a tool to estimate the effects of potential confounders on birthweight. Infant gender, gestational age and maternal BMI are all significantly correlated with birthweight, which is consistent with previous literature reports [31]. Maternal smoking has been reported to be a confounding factor that is significantly associated with singleton birthweight, which was not demonstrated in our study [32, 33]. This is likely attributed to the fact that the majority of our participants recruited in the trial were nonsmokers, therefore the power to detect such an effect was insufficient, or it could be due to the freeze-thaw process itself.

This study was limited by its retrospective nature and an inherent selection bias. To eliminate or minimize potential confounders related to newborn birthweight, we rigorously reviewed the data with strict criteria to eliminate possible confounders. Most importantly, in our research, IVF laboratory procedures, embryo culture conditions, culture media, and laboratory consumables were constant during the study period.

In conclusion, our retrospective study showed that post-thaw culture duration of thawed cleavage-stage embryos did not affect the birthweight of singletons in FET cycles, but the proportion of SGA infants was significantly higher in the long culture duration group after an HRT. As a result, both culture procedures can be applied well, and the decision would depend on the laboratory workflow. To further confirm our conclusions, a well-designed multi-center prospective randomized controlled clinical trial (RCT) should be performed.

### Funding

No external funding.

### Ethical approval

Not applicable.

### Conflict of interest

The authors declare no conflict of interest.

### References

1. Adamson GD, de Mouzon J, Chambers GM, et al. International Committee for Monitoring Assisted Reproductive Technology: world report on assisted reproductive technology, 2011. *Fertil Steril* 2018; 110: 1067-80.
2. Doody KJ. Cryopreservation and delayed embryo transfer-assisted reproductive technology registry and reporting implications. *Fertil Steril* 2014; 102: 27-31.
3. Rato ML, Gouveia-Oliveira A, Plancha CE. Influence of post-thaw culture on the developmental potential of

- human frozen embryos. *J Assist Reprod Genet* 2012; 29: 789-95.
4. Wang H, Ou Z, Chen Z, et al. Influence of different post-thaw culture time on the clinical outcomes of different quality embryos. *Adv Clin Exp Med* 2019; 28: 523-7.
  5. Ji H, Cao S, Ding H, et al. Influence of post-thaw culture duration on pregnancy outcomes in frozen blastocyst transfer cycles. *Syst Biol Reprod Med* 2022; 69: 64-74.
  6. Agha-Rahimi A, Omid M, Akyash F, et al. Does overnight culture of cleaved embryos improve pregnancy rate in vitrified-warmed embryo transfer programme? *Malaysian J Med Sci* 2019; 26: 52-8.
  7. Sordia-Hernandez LH, Martinez FAM, Orozco EG, et al. The effect of post warming culture period between thawing and transfer of cryopreserved embryos on reproductive outcomes after in vitro fertilization (IVF): a systematic review and meta-analysis. *J Reprod Infertil* 2021; 22: 77-84.
  8. Pham HH, Vu TM, Nguyen CH, et al. Effect of post-warming culture time on the live birth rate after frozen embryo transfer. *Reprod Med Biol* 2022; 21: e12465.
  9. Zhu H, Xu W, Jin X, et al. Association of the duration of post-thaw culture with clinical outcome after vitrified-warmed day 3 embryo transfer in 10,464 cycles: a retrospective cohort study. *Medicine* 2020; 99: e21660.
  10. Kuwayama M, Vajta G, Kato O, et al. Highly efficient vitrification method for cryopreservation of human oocytes. *Reprod Biomed Online* 2005; 11: 300-8.
  11. Hu KL, Zhang D, Li R. Endometrium preparation and perinatal outcomes in women undergoing single-blastocyst transfer in frozen cycles. *Fertil Steril* 2021; 116: 1487-94.
  12. Herman HG, Mizrachi Y, Alon AS, et al. Obstetric and perinatal outcomes of pregnancies resulting from fresh versus frozen embryo transfer-a sibling cohort. *Reprod Sci* 2022; 29: 1644-50.
  13. Schisterman EF, Whitcomb BW, Mumford SL, et al. Z-scores and the birthweight paradox. *Paediatr Perinat Epidemiol* 2009; 23: 403-13.
  14. Dai L, Deng C, Li Y, et al. Birth weight reference percentiles for Chinese. *PLoS One* 2014; 9: e104779.
  15. Maheshwari A, Raja EA, Bhattacharya S. Obstetric and perinatal outcomes after either fresh or thawed frozen embryo transfer: an analysis of 112,432 singleton pregnancies recorded in the Human Fertilisation and Embryology Authority anonymized dataset. *Fertil Steril* 2016; 106: 1703-8.
  16. Palomba S, Homburg R, Santagni S, et al. Risk of adverse pregnancy and perinatal outcomes after high technology infertility treatment: a comprehensive systematic review. *Reprod Biol Endocrinol* 2016; 14: 76.
  17. Watkins WJ, Kotecha SJ, Kotecha S. All-cause mortality of low birthweight infants in infancy, childhood, and adolescence: population study of England and Wales. *PLoS Med* 2016; 13: e1002018.
  18. Romundstad LB, Romundstad PR, Sunde A, et al. Effects of technology or maternal factors on perinatal outcome after assisted fertilisation: a population-based cohort study. *Lancet* 2008; 372: 737-43.
  19. Sunde A, Brison D, Dumoulin J, et al. Time to take human embryo culture seriously. *Hum Reprod* 2016; 31: 2174-82.
  20. Makinen S, Soderstrom-Anttila V, Vainio J, et al. Does long in vitro culture promote large for gestational age babies? *Hum Reprod* 2013; 28: 828-34.
  21. Pereira N, Elias RT, Christos PJ, et al. Supraphysiologic estradiol is an independent predictor of low birth weight in full-term singletons born after fresh embryo transfer. *Hum Reprod* 2017; 32: 1410-7.
  22. Zhang J, Wang Y, Liu HF, et al. Effect of in vitro culture period on birth weight after vitrified-warmed transfer cycles: analysis of 4,201 singleton newborns. *Fertil Steril* 2019; 111: 97-104.
  23. De Vos A, Santos-Ribeiro S, Van Landuyt L, et al. Birthweight of singletons born after cleavage-stage or blastocyst transfer in fresh and warming cycles. *Hum Reprod* 2018; 33: 196-201.
  24. Murakami M, Tanaka K, Otsubo H, et al. Differential impact of three embryo culture media for IVF on in vitro development and perinatal outcome: a single-center RCT. *Hum Reprod* 2021; Suppl 1: P-146.
  25. Gu F, Deng M, Gao J, et al. The effects of embryo culture media on the birthweight of singletons via fresh or frozen-thawed embryo transfer: a large-scale retrospective study. *BMC Pregnancy Childbirth* 2016; 16: 270.
  26. Bick L, Nielsen AS, Knudsen UB. Embryo culture media influence on live birth rate and birthweight after IVF/ICSI: a systematic review comparing Vitrolife G5 Media to Other Common Culture Media. *JBRA Assist Reprod* 2021; 25: 480-92.
  27. Fang J, Zhu L, Li D, et al. Effect of embryo and blastocyst transfer on the birthweight of live-born singletons from FET cycles. *J Assist Reprod Genet* 2018; 35: 1905-10.
  28. Wang B, Zhang J, Zhu, et al. Effects of different cycle regimens for frozen embryo transfer on perinatal outcomes of singletons. *Hum Reprod* 2020; 35: 1612-22.
  29. Bonagura TW, Pepe GJ, Enders AC, et al. Suppression of extravillous trophoblast vascular endothelial growth factor expression and uterine spiral artery invasion by estrogen during early baboon pregnancy. *Endocrinology* 2008; 149: 5078-87.
  30. Li C, He YC, Xu JJ, et al. Perinatal outcomes of neonates born from different endometrial preparation protocols after frozen embryo transfer: a retrospective cohort study. *BMC Pregnancy Childbirth* 2021; 21: 341.
  31. Ma M, Zhang W, Zhang J, et al. Effect of paternal body mass index on neonatal outcomes of singletons after frozenthawed embryo transfer cycles: analysis of 7,908 singleton newborns. *Fertil Steril* 2020; 113: 1215-23.
  32. Maas K, Galkina E, Thornton K, Penzias AS, Sakkas D. No change in live birthweight of IVF singleton deliveries over an 18-year period despite significant clinical and laboratory changes. *Hum Reprod* 2016; 31: 1987-96.
  33. Wojtyla C, Wojtyla-Buciora P, Ciebiera M, Orzechowski S, Wojtyla A. The effect of active and passive maternal smoking before and during pregnancy on neonatal weight at birth. *Arch Med Sci* 2021; 17: 352-60.