Original Article
Effects of different endometrial preparation on birthweight of frozen-thawed embryo transfer

Jinli Ding*, Yi Zhang*, Tailang Yin, Jing Yang

*Reproductive Medicine Center, Renmin Hospital of Wuhan University, Wuhan, Hubei Province, China. *Equal contributors.*

Abstract: Purposes: To evaluate whether different endometrial preparation have an impact on neonatal birth weight of frozen-thawed embryo transfer. Methods: This was a retrospective study in a university teaching hospital in Wuhan, China from January, 2010 to March, 2013. A total of 528 women receiving frozen-thawed embryo transfer were included in the study, and they were divided into two groups according to the endometrial preparation, namely natural cycle and stimulated cycle. The newborn outcomes were investigated and compared. Results: There were no significant differences in gestational age, average birth weight, preterm birth rate, very preterm birth rate, low birth weight and very low birth weight between natural cycle and stimulated cycle of FET. Multiple linear regression analysis showed that birth weight was associated with gestational age, number of newborn and gender. Conclusions: Different endometrial preparation has no difference on neonatal birthweight, regardless of singletons or twins.

Keywords: Natural cycle, stimulated cycle, birthweight, gestational age

Introduction

Cryopreservation of embryos has been a well-established part of assisted reproduction technology (ART) with increasing delivery rates and widely used in assisted reproduction center since frozen-thawed embryo transfer (FET) has been successfully performed during a natural cycle after spontaneous ovulation or stimulated preparation of endometrium [1-6], which provides the possibility of further embryo transfers to achieve clinical pregnancy. FET plays an important role in improving cumulative pregnancy rate and reducing ovarian hyperstimulation [6, 7].

Factors effecting the success rate of FET cycle include the quality of the frozen embryo, the stage of the embyro at freezing, the survival rate after thawing and the number of the embryo transferred, storage duration and the technique of the operator [8-12], and numerous studies have shown that freezing programme used, maternal age, fresh embryo or frozen embryo, chronic disease, smoking sta-

tus [10, 13-17] have great effects on birth weight. A significant factor for implantation successfully and subsequent pregnancy outcome in FET was the synchronization between embryo development and endometrial maturation. The influence of the number of implantation, embryo culture and type of embryos transferred on perinatal outcome have been reported by many authors. Differences exist in the pregnancy outcome of differernt endometrial preparation [18-21]. But whether different endometrial preparation has various effects on birth weight or gestational age of FET? The aim of this retrospective study was to evaluate the effects of different endometrial preparation on birth weight of FET. The hypothesis was that natural cycle was similar to stimulated cycle on the pregnancy outcomes.

Materials and methods

Study population

Seven hundred and forty-four women who failed to conceive in their fresh IVF cycle and under-

Table 1. Demographic information of patients

Cycles (n=number)	Natural cycle (146)	Stimulated cycle (382)	P value
Maternal age (year)	30.84±4.36	30.63±4.39	0.62
Infertility duration (year)	4.45±3.09	4.65±3.15	0.50
Body mass index (kg/m²)	21.69±3.25	21.98±3.07	0.35
Causes of infertility			
Female factor	74.66 (109)	79.32 (303)	0.15
Male factor	39.73 (58)	37.70 (144)	0.37
Undefined factor	0.68 (5)	2.09 (8)	0.28
Primary infertility	44.52 (65)	50.00 (191)	0.15
Number of embryo transferred	2.21±0.44	2.16±0.43	0.23
Gestational age (day)	266.05±10.80	264.37±14.27	0.20

went the first FET at Renmin Hospital of Wuhan University from January 2010 to March 2013, and obtained clinical pregnancy were recruited. Clinical pregnancy was confirmed as gestational sac observation under the transvaginal ultrasonographic at 5 weeks. Among them, thirty-two patients were lost to follow-up, twenty-four patients were diagnosed hypertensive disorders in pregnancy, twenty were diabetes, twenty-four were ectopic pregnancy, one patient induced because of fetal congenital heart disease, and one hundred and fifteen women miscarried. Only five hundred and twenty-eight women gave birth to singletons or twins successfully. The study protocol was approved by the Institutional Review Board of Renmin Hospital of Wuhan University.

Treatment regimen

In this study, 0.1 mg/d GnRH-a was administrated at the mid-luteal phase of the previous menstrual cycle, and ovarian stimulation was started 14 days later with a starting dose varying from 75 to 300 IU/day re-combinant FSH according to patients' ovarian reserve and age. 8 000~10 000 IU hCG was administered to induce final oocyte maturation when there were three or more larger follicle reached 18 mm in diameter. Oocyte retrieval was performed 36 hours later, and fertilization was conducted according to standard procedures [22]. Good-quality embryo transplantation was performed 3 days after oocyte retrieval, which was assessed according to the Society for Assisted Reproductive Technology embryo grading system [23]. Surplus embryos of good quality were cryopreserved by vitrification. The storage dura-

tion of frozen-thawed embryo was less one year, and all were the first time thawing. On the day of FET, the embryos were thawed rapidly and then transferred to culture medium. The vitrification and thawing were performed according to the instruction (Vitrification Media VT101, Thawing Media VT102, Kitazato BioPharma Co. Ltd., Shizuoka, Japan), as described previously [18].

For women with regular ovulatory cycles, FET was performed in natural cycle. Patients were instructed to monitor urine LH 10th day in their menstruation. When ovulation was confirmed, ultrasound scan was conducted to confirm ovulation and endometrial development. Intramuscular injection progesterone in oil 40 mg/d was administered for all patients 1 day after ovulation, FET was carried out 3 day after the start of progesterone administration. Patients with irregular menstruation were chosen for the stimulated cycle. Estradiol valerate tablet (Progynova Bayer Schering Pharma, Guangzhou China) was commenced orally 2 mg/d from day 3 to day 5, 4 mg/d from day 6 to day 9 and 6 mg/d from day 10 to the day of the pregnancy test. Then, progesterone in oil was administered 40 mg/d when the endometrial thickness ≥8 in millimeter. Also, FET was carried out 3 day after the start of progesterone, and then progesterone in oil was commenced 60 mg/d until the day of the pregnancy test. 2~3 embryos were transferred in each FET cycle depending on the number of preceding cycles and maternal age.

Outcome measures

The outcome of the study was gestational age and average birth weight. Preterm birth was birth < 37 weeks of gestation and very preterm birth < 32 weeks of gestation. Low birth weight was defined as birth weight < 2500 g and very low birth weight as < 1500 g.

Statistical analysis

Statistical analysis was performed by Statistical Package for Social Sciences (SPSS) software. The basic information of patients and newborns were compared by analysis of variables

Table 2. Information of singletons

	Natural cycle (104)	Stimulated cycle (279)	P value
Maternal age (year)	30.82±4.36	30.36±4.39	0.36
Infertility duration (year)	4.46±2.98	4.50±3.04	0.91
Body mass index (kg/m^2)	21.61±3.13	21.94±3.02	0.35
Gestational age (days)	270.04±9.13	268.58±11.35	0.24
Boy (192)	268.56±10.38 (n=52)	267.30±12.16 (n=140)	0.51
Girl (191)	271.52±7.51 (n=52)	269.88±10.36 (n=139)	0.30
Birth weight	3413.65±443.54	3387.92±536.85	0.66
Boy	3431.15±510.13	3409.00±547.13	0.80
Girl	3396.15±369.40	3366.69±527.40	0.71
Preterm birth	5.77 (6)	8.24 (23)	0.28
Very preterm birth	0.00 (0)	0.72 (2)	0.53
Low birth weight	2.89 (3)	5.02 (14)	0.28
Very low birth weight	0.00 (0)	0.00 (0)	-

Preterm: gestational age < 37 weeks; Very preterm: gestational age < 32 weeks; Low birth weight: birth weight < 2500 g; Very Low birth weight: birth weight < 1500 g.

Table 3. Information of twins

	Natural cycle (42)	Stimulated cycle (103)	P value
Maternal age (year)	30.91±4.39	31.37±4.35	0.56
Body mass index (kg/m^2)	21.89±3.58	22.09±3.20	0.29
Infertility duration (year)	4.43±3.37	5.08±3.39	0.74
Gestational age (day)	256.17±7.89	252.95±15.12	0.19
Boy (37)	256.08±6.97 (n=12)	248.08±15.00 (n=25)	0.09
Girl (38)	260.00±7.48 (n=7)	254.48±13.71 (n=31)	0.31
Pigeon Pair (70)	255.04±8.40 (n=23)	254.53±15.82 (n=47)	0.89
Total birth weight (g)	5279.05±594.51	5209.85±1017.98	0.68
Girl (38)	5333.33±415.24	5300.00±982.56	0.91
Boy (37)	5414.29±698.04	5074.03±1154.80	0.46
Pigeon Pair (70)	5209.57±653.53	5251.50±951.75	0.85
Preterm birthrate	45.24 (19)	43.69 (45)	0.50
Very preterm birth	0.00 (0)	0.00 (0)	0.18
Low birth weight	33.33 (14)	28.16 (29)	0.33
Very low birth weight	0.00 (0)	4.85 (5)	0.18

Preterm: gestational age < 37 weeks; very preterm: gestational age < 32 weeks; low birth weight: total birth weight < 5000 g; very low birth weight: total birth weight < 3000 g.

(ANOVA) and categorical variables were evaluated by χ^2 tests. Multiple linear regression analysis was used to evaluate the association between different cycles and birthweight. Value of $P < 0.05$ was considered statistically significant.

Results

In the natural cycle group, 146 FET were included, and 382 FET were included in the stimulated cycle group. The demographic data and infertility characteristics of both groups were not significantly different (**Table 1**).

Data on singletons in terms of birth weight, gestational age of both natural cycle and stimulated cycle was detailed in **Table 2**. There was no significant difference in terms of maternal age, body mass index and infertility duration. The preterm delivery rate was 7.57% (29/383), very preterm birth rate 0.52% (2/383) and low birth weight 4.44% (17/383). No very low birth weight was noticed in both group, and no very preterm

Table 4. Multiple linear regression analysis

	Birth weight			
	B	Beta	t	P value
Maternal age	3.277	0.023	0.881	0.38
Infertility duration	-6.642	-0.330	-1.278	0.20
Infertility type	17.002	0.013	0.522	0.60
Body mass index	5.920	0.029	1.147	0.25
Endometrial preparation	22.715	0.016	0.629	0.53
Gestational age	25.199	0.538	17.923	0.00
Genger	124.870	0.098	3.865	0.00
Number of newborn	-378.892	-0.294	-9.003	0.00

birth was noticed in the natural cycle group in contrast to the stimulated cycle group (0.00% vs. 0.72%, P=0.53). The comparison of gestational age (270.04±9.13 vs. 268.58±11.35, P=0.24), birth weight (3413.65±443.54 vs. 3387.92±536.85, P=0.66), preterm birth (5.77% vs. 8.24%, P=0.28) and low birth weight (2.89% vs. 5.02%, P=0.28) showed no statistical difference between the two groups. The newborn sex ratio for all singletons was 192:191, although a trend of higher gestational age for girls was observed, statistical significance was not reached (natural cycle, 268.56 ±10.38 vs. 271.52±7.51, P=0.098; stimulated cycle, 267.30±12.16 vs. 269.88±10.36, P=0.058). No statistical significance was observed in the birth weight between boys and girls (natural cycle, 3431.15±510.13 vs. 3396.15± 369.40, P=0.689; stimulated cycle, 3409.00± 547.13 vs. 3366.69±527.40, P=0.511).

Table 3 showed the information of twins. There was no significant difference in terms of maternal age, body mass index and infertility duration. The preterm delivery rate for twins was 44.14% (64/145), low birth weight 29.66% (43/145) and very low birth weight 3.45% (5/145). No very preterm birth was noticed in both group, and no very low birth weight was noticed in the natural cycle group in contrast to the stimulated cycle group (0.00% vs. 4.85%, P=0.18). When comparing the outcomes of FET, we found no significant difference in total birth weight, preterm birth rate, low birth weight between two groups. The newborn sex ratio for twins was 144:146 (boys group, 37; girls group, 38; pigeon Pairs group, 70), no statistical significance was observed for gestational age (χ^2=1.46, P=0.24) and birth weight (χ^2=0.34, P=0.71) between three groups.

Finally, multiple linear regression analysis was used to determine the relationship between birth weight and endometrial preparation cycle, maternal age, BMI, causes of infertility, types of infertility, gestational age and neonatal gender. As shown in **Table 4**, birth weight was associated with gestational age, number of newborn and gender.

Discussion

This restrospective study tested an idea regarding the effect of different endometrial preparation on the newborn outcomes. Our retrospective analysis showed that different endometrial preparation does not significantly influence the neonatal birth weight after FET, and this finding was supported by multiple linear analyses. To our knowledge, this was the first study focused on the effect of endometrial preparation on the newborn outcomes.

FET has become an effective way to improve pregnancy outcome, avoiding the presence of severe OHSS and reducing the cost of ART since it was first reported in 1983. Protocols for endometrial preparation in FET include the natural cycle and the stimlated cycle. There has been controversy regarding the effect of different endometrial preparation on pregnancy outcome [18-20]. The natural cycle is a simple and cost-effective protocol for FET for women with regular and ovulatory cycles, but it is tedious to monitor the endometrium to detect the onset of the LH surge and guarantee the precise synchronization for most women. While, the simulated cycle is preferred because it was easier to determine the time of transplant, which could reduce the cancellation rate. Unfortunately, it's high cost, which makes it unaccepted despite of its effectiveness and safety for some women. In natural cycle, the endocrine preparation is achieved by endogenous sex steroid production from a developing follicle and in stimlated cycle, progesterone and estrogen are administered in a regimen which aims to imitate the endocrine environment of the endometrium as the normal cycle. Some authors of early reports suggests that natural FET cycles have higher implantation and pregnancy rates compared with stimulated FET cycles [18], whereas others report no such effects [19-21].

In our study, multiple linear regression analysis showed that birth weight was associated with the number of newborn, which was in line with accumulating reports showing that a multiple pregnancy is regarded to be one of the factors leading to preterm birth as well as to low birth weight [9, 24-26]. Our results also showed that the pregnancy length was associated with birth weight, which was consistent with earlier studies that preterm birth increased the the risk of low birth weight [27, 28]. ART newborns are reported to have an increased risk of being small for gestational age, preterm birth and low birth weight [24, 25]. In this study, the rates of low birth weight were 4.44% for singletons and 29.66% for twins (P=0.00), and the rates of preterm birth were 7.57% (29/383) for singletons and 44.14% (64/145) for twins (P=0.00), these outcomes were similar to the results from earlier studies [17, 24, 25]. The total rate of low birth weight was 15.89% (107/673), which was similar to 15.5% of all birth infants worldwide [29].

It was important to adjust the absolute birth weight at least for gestational age and newborn gender when reporting birth weight, and, some confounding factors should be considered, including maternal age, duration and cause of infertility, maternal smoking [13, 16, 17]. Chronic diseases, such as hypertensive disorders and diabetes, had been shown to be related with birth weight, which may affect fetal development, and be associated with reduced fetal growth [15, 30], so, we eliminated these patients in our study. Smoking during pregnancy was related to infant birth weight. Few women smoke in the People's Republic of China, especially during pregnancy, so smoking history was not included in our analysis.

No statistical difference was observed in the birth weight of different gender in singletons or twins, when ignoring singletons or twins, a trend of higher birth weight for boys was observed (3092.23±630.09 vs. 3002.63± 626.04, P=0.063) (date was not shown in the table), and the multiple linear regression analysis showed that gender was associated with birth weight (P=0.00).

According to Nelson et al, a higher maternal age increased the risk of low birth weight [13], our study showed that there was no correlation between maternal age and birth weight, which

was similar to the results with earlier study [31]. It was probably because pregnant women following ART treatment were motivated to reduce daily activity, which contributing to a decrease in the rate of low birth weight in older women [31].

A meta-analysis revealed no significant difference of one specific approach to prepare the endometrium for FET in terms of clinical pregnancy rates or live birth rates [21], and our results showed that there was no differerce for different endometrial preparation on the newborn outcomes. Since no significant difference in the pregnancy rate and newborn outcomes was found, the choice for either stimulated cycle or natural cycle in FET should be made based on other factors, such as doctor or patient preference, side-effects of medication, the number of FET, the endometrial environment and the number of canceled cycles.

The main limitation of this study was the absence of the long-term follow-up data of newborns and the limited sample size. So, long-term and more large sample researches were needed.

In conclusion, neonatal birth weight is not influenced by the different endometrial preparation included in our study. However, the influence of different endometrial preparation on the newborn outcomes still needs further exploration.

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Disclosure of conflict of interest

None.

Address correspondence to: Jing Yang, Reproductive Medicine Center, Renmin Hospital of Wuhan University, Wuhan, Hubei Province, China. E-mail: dryangqing@hotmail.com

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