The likelihood of a healthy live birth after frozen embryo transfer with endometrium prepared by natural ovulation regimen vs programmed regimen: a propensity-score matching study

Ze Wang, MD; Yingxin Zhang, PhD; Xue Shang, MS; Ruolan Miao, MS; Mengfei Yin, MS; Huiming Yang, MS; Yunhai Yu, MD; Daimin Wei, PhD, MD

BACKGROUND: The number of frozen embryo transfer cycles is increasing, but the optimal method of endometrial preparation for frozen embryo transfer remains controversial. Few studies have investigated the healthy live birth outcome after the natural ovulation regimen vs the programmed regimen.

OBJECTIVE: This study aimed to explore whether the likelihood of a healthy live birth after frozen embryo transfer differs between the natural ovulation regimen and the programmed regimen.

STUDY DESIGN: We conducted a retrospective cohort study including 7824 ovulatory women who underwent the first frozen embryo transfer cycle of single-blastocyst transfer with endometrial preparation by natural ovulation regimen vs programmed regimen, between June 2017 and June 2021. Propensity score matching was used to control for confounding variables in a 1:1 ratio. The primary outcome was healthy live birth, defined as birth of a live, singleton infant born at term, with an appropriate birthweight for gestational age.

RESULTS: The natural ovulation regimen resulted in a higher probability of achieving healthy live birth compared with the programmed regimen (35.8% vs 30.6%; $P<.0001$). In addition, a higher rate of singleton live birth was observed after the natural ovulation regimen relative to the programmed regimen (49.6% vs 45.7%; $P=.003$). Women with the natural ovulation regimen were also less likely to experience clinical pregnancy loss (16.0% vs 19.7%; $P=.005$) and hypertensive disorders of pregnancy (3.9% vs 6.0%; $P=.004$) compared with women with the programmed regimen. Singletons born after the programmed regimen had greater mean birthweight (3441.50±539.97 vs 3394.96±503.87; $P=.020$) and higher risk of being large for gestational age (23.3% vs 18.7%; $P=.003$) than those conceived after the natural ovulation regimen.

CONCLUSION: The natural ovulation regimen may be superior to the programmed regimen with regard to higher likelihood of healthy live birth and lower risk of pregnancy loss and maternal hypertensive disorders of pregnancy.

Key words: corpus luteum, frozen embryo transfer, healthy live birth, programmed regimen, propensity score

Introduction

Frozen embryo transfer (FET) was initially invented to cryopreserve the supernumerary embryos after fresh embryo transfer. The introduction of vitrification and the practice of single-embryo transfer have contributed to an expanding number of FET cycles. Moreover, randomized clinical trials and meta-analyses have demonstrated equivalent or higher live birth rate following the "freeze-all" policy relative to conventional fresh embryo transfer,[1–4] thus further boosting the rapid growth of FET cycles. However, compared with fresh embryo transfer, the increased risk of maternal pre-eclampsia and the higher probability of large-for-gestational-age (LGA) infants emerged as concerns regarding the safety of FET.[5] Nonetheless, the

From the Center for Reproductive Medicine, Shandong University, Jinan, China (Dr Wang, Ms Shang, Dr Miao, Dr Yin, Dr Yang, and Dr Wei); Department of Clinical Laboratory, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, China (Dr Zhang); Department of Obstetrics and Gynecology, Second Hospital, Cheeloo College of Medicine, Shandong University, Jinan, China (Dr Yu).

Z.W. and Y.Z. share first authorship.

The authors report no conflict of interest.

This study was supported by the National Natural Science Foundation of China (82071718) and the Natural Science Foundation of Shandong Province (ZR2021MH391 and ZR2021QH320).

All participants provided written informed consent.

Cite this article as: Wang Z, Zhang Y, Shang X, et al. The chance of a healthy live birth after frozen embryo transfer with endometrium prepared by natural ovulation regimen vs programmed regimen: a propensity-score matching study. Am J Obstet Gynecol Glob Rep 2023;XX:x.ex–x.ex.

Corresponding author: Daimin Wei, MD, PhD. sdweidaimin@163.com

2666-5778/$36.00

http://dx.doi.org/10.1016/j.xagr.2023.100210

AJOG Global Reports at a Glance

Why was this study conducted?

This study aimed to assess the healthy live birth outcome after frozen embryo transfer (FET) with endometrium prepared by the natural ovulation regimen vs the programmed regimen.

Key findings

The natural ovulation regimen for endometrial preparation before FET was associated with a higher likelihood of healthy live birth compared with the programmed regimen.

What does this add to what is known?

The natural ovulation regimen for endometrial preparation before FET may be superior to the programmed regimen from a holistic view of efficacy and safety.

underlying mechanism was still unclear.

The natural ovulation regimen and the programmed regimen are the mainstays of endometrial preparation methods for FET. Natural ovulation regimen requires close monitoring of follicular growth until spontaneous or triggered ovulation, whereas programmed regimen mimics the proliferative- and secretory-phase endometrium by consecutive administration of exogenous estrogen and progesterone without formation of the corpus luteum (CL). The optimal method of endometrial preparation for FET remains controversial. The most recent Cochrane reviews pooling randomized trials of natural ovulation regimen vs programmed regimen yielded inconclusive results in terms of pregnancy and live birth.[6,7] Conversely, recent observational studies found that the programmed regimen was associated with higher risks of maternal preeclampsia and delivering LGA infants when compared with the natural ovulation regimen.[8,9]

The ultimate goal of in vitro fertilization (IVF) treatment is to achieve a healthy live birth while minimizing the risk of maternal complications from a holistic view of efficacy and safety. A healthy live birth is generally defined as birth of a live, singleton infant born at term with an appropriate birthweight for gestational age.[10] It is well-known that preterm birth is the leading cause of perinatal mortality and morbidity.[11] Abnormal fetal growth, commonly defined as small-for-gestational-age (SGA) or LGA, is also linked to elevated risk of perinatal complications and has long-term implications for offspring health.[12,13] Yet, few studies have investigated the healthy live birth outcome after different methods of endometrial preparation for FET.

The primary purpose of this study was to explore whether the likelihood of having a healthy live birth differed between natural ovulation regimen and programmed regimen after controlling for potential confounders using a propensity score (PS) matching design.

Materials and Methods

Study design and population

This was a retrospective cohort study conducted in a single university-affiliated reproductive center. Ovulatory women undergoing the first FET cycle of single-blastocyst transfer between June 2017 and June 2021 were included. Either the natural ovulation regimen or programmed regimen was used to prepare the endometrium. Ethical approval for this study was granted by the ethics committee of the hospital (ethical approval number: 2022-35). Anonymous data were extracted from the electronic medical record system. The exclusion criteria were as follows: (1) presence of uterine abnormalities including uterine congenital abnormalities and untreated submucosal myoma, endometrial polyps, and intrauterine adhesions; (2) women with recurrent pregnancy loss; (3) women who underwent preimplantation genetic testing; and (4) embryos derived from oocyte donation or oocyte cryopreservation or having undergone thawing and recryopreservation process.

Endometrial preparation regimens

Because no consensus has been reached on the optimal method of endometrial preparation for FET, the decision of natural ovulation regimen or programmed regimen for endometrial preparation was mainly dependent on clinician discretion and/or patient preference.

Natural ovulation regimen. A transvaginal ultrasound scan was started on menstrual day 9 to 11 depending on menstrual cycle length, and subsequently repeated every 1 to 3 days according to the growing speed of the dominant follicle. Ovulation occurred spontaneously, and the day of ovulation was determined by ultrasonographic signs of ovulation event, with or without measurement of progesterone. Single-blastocyst transfer was performed 5 days after ovulation. Oral 10-mg dydrogesterone 2 or 3 times daily was initiated after ovulation. If pregnancy was confirmed, luteal phase support was continued until 10 to 11 weeks of gestation.

Programmed regimen. Oral estradiol (estradiol valerate or 17-beta-estradiol) with the priming dosage of 4 to 6 mg daily was started on days 1 to 4 of the menstrual cycle. After 10 to 15 days, transvaginal ultrasound was performed to measure the endometrial thickness and exclude the presence of a dominant follicle, while endocrine measurement of serum estradiol and progesterone levels was taken. Estrogen types, route, and dosages could be adjusted if required. When the endometrial thickness reached at least 8 mm and the serum progesterone level was <1.5 ng/mL, administration of vaginal-micronized 200-mg per day progesterone combined with 20-mg oral dydrogesterone twice daily was initiated. Embryo transfer was performed on the sixth day of progesterone initiation. If pregnancy occurred,

estrogen supplementation was stopped at 7 to 8 weeks of gestation, and progesterone support was continued until 10 to 11 weeks of gestation.

Outcome measures

The primary outcome was a healthy live birth, defined as a singleton live birth delivered at ≥37 weeks with normal birthweight (between the 10th and 90th percentile for gestational age).

The secondary outcomes included live birth (defined as delivery of ≥1 viable neonate at ≥28 weeks of gestation), clinical pregnancy (defined as detection of ≥1 gestational sacs in the uterus by ultrasonography), clinical pregnancy loss (defined as spontaneous ending or therapeutic abortion of clinical pregnancy before 28 weeks of gestation), hypertensive disorders of pregnancy (HDP), gestational diabetes mellitus, preterm delivery (defined as delivery at <37 weeks of gestation), birthweight, low birthweight (<2500 g), macrosomia (birthweight >4000 g), and SGA and LGA. SGA and LGA in singletons were defined as birthweight <10th and >90th percentile of the reference birthweight percentiles for Chinese singletons, respectively, after adjusting for gestational age and neonatal sex.[14]

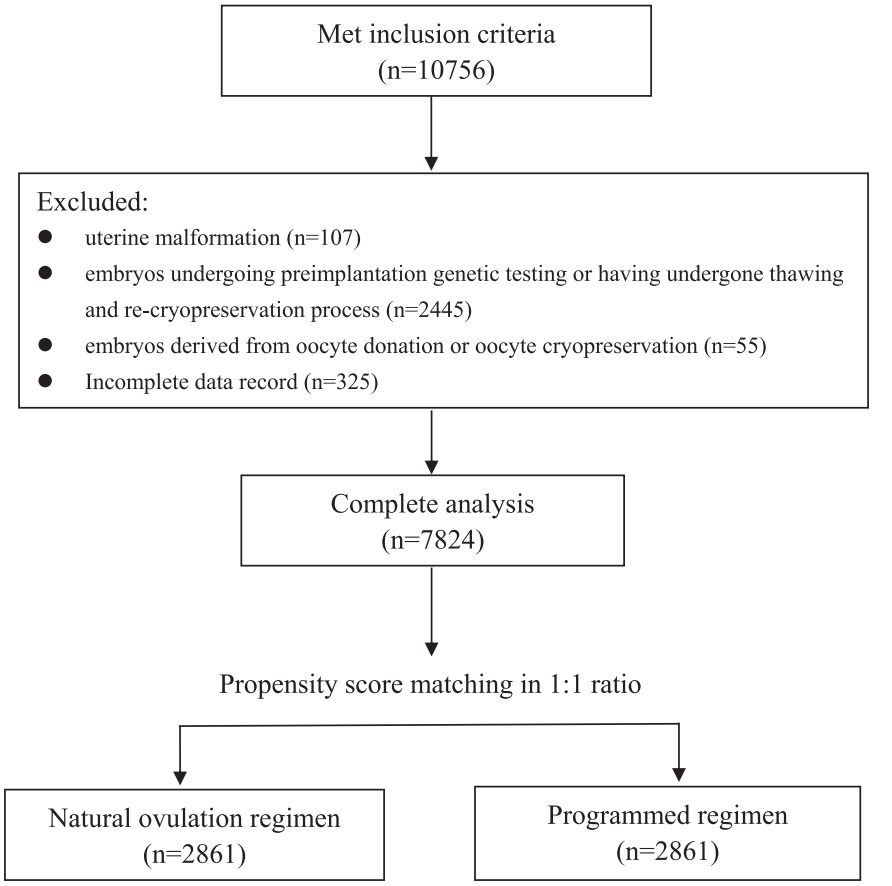
Statistical analysis

PS matching analysis was used to minimize the effects of confounding factors and selection bias because of the retrospective nature of this study. Patients in the programmed regimen group were matched in a 1:1 ratio with patients in the natural ovulation regimen group, using nearest-neighbor matching within a caliper of 0.02, without replacement. The variables used for matching included maternal age (continuous), body mass index (BMI) (continuous), gravidity (0 vs ≥1), parity (0 vs ≥1), history of uterine adhesion (0 vs ≥1), causes of infertility (tubal factors vs male factors vs others), total testosterone level (continuous), anti-Müllerian hormone level (AMH) (continuous), total antral follicle count (AFC) (continuous), fasting glucose level (continuous), protocols for controlled ovarian hyperstimulation (gonadotropin-releasing hormone [GnRH]-agonist long vs GnRH-antagonist vs GnRH-agonist short vs others), total gonadotropin dose (continuous), endometrial thickness on human chorionic gonadotropin (hCG) trigger day (continuous), number of retrieved oocytes (continuous), fertilization method (IVF vs intracytoplasmic sperm injection), and developmental stage of transferred embryo (D5 vs D6 vs D7). Data were presented as means±standard deviations for normally distributed continuous variables, median (interquartile range) for nonnormally distributed continuous variables, and frequencies (percentages) for categorical variables. Comparisons between groups were carried out using the Student *t* test or Mann−Whitney *U* test for continuous variables, and chi-square or Fisher exact test for categorical variables. Balance in the baseline covariates between groups after matching was assessed using standardized differences. An absolute standardized difference of <0.1 was considered to indicate a good balance. PS matching was performed using the MatchIt package in R, version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria). Statistical comparisons were performed using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp, Armonk, NY). A *P* value (2-sided) of <.05 indicated statistical significance.

Results

Figure 1 shows the flow diagram of the study population. Overall, there were 4690 women in the natural ovulation regimen group and 3134 women in the programmed regimen group. Baseline characteristics before PS matching are

FIGURE 1
Flowchart of the study population



Wang. Healthy live birth outcome after frozen embryo transfer prepared by natural ovulation vs programmed regimen. Am J Obstet Gynecol Glob Rep 2023.

summarized in Table 1. Compared with women with the natural ovulation regimen, women with the programmed regimen were younger and had higher BMI, AFC, and total testosterone and AMH levels. Women with no history of childbirth and women with a history of uterine adhesion were more common in the programmed regimen group than in the natural ovulation regimen group. A higher dosage of total gonadotropins and thinner endometrium on the day of hCG trigger were observed in women with the programmed regimen compared with women with the natural ovulation regimen. There was no significant difference between the 2 groups in duration of infertility, proportion of primary infertility, number of previous miscarriages, follicle-stimulating hormone level, fasting glucose level, estradiol level on the day of hCG trigger, number of retrieved oocytes, fertilization method, or the developmental stage of embryo transferred. After PS matching, the natural ovulation regimen group consisted of 2861 women matched to 2861 women in the programmed regimen group. The baseline characteristics were well-balanced between the matched groups (Table 1; Supplementary Table I).

The pregnancy and neonatal outcomes are listed in Table 2. The natural ovulation regimen resulted in a higher probability of achieving healthy live birth compared with the programmed regimen (35.8% vs 30.6%; $P<.0001$). A total of 60.3% of women in the natural ovulation regimen group and 57.9% in the programmed regimen group achieved clinical pregnancy, with no statistically significant difference ($P=.075$). A higher rate of singleton live birth was observed after the natural ovulation regimen relative to the programmed regimen (49.6% vs 45.7%; $P=.003$). Women with the natural ovulation regimen were less likely to experience clinical pregnancy loss (16.0% vs 19.7%; $P=.005$) and HDP (3.9% vs 6.0%; $P=.004$) compared with women with the programmed regimen. Singletons born after the programmed regimen had a greater mean birthweight (3441.50±539.97 vs 3394.96±503.87; $P=.020$) and higher risk of being LGA (23.3% vs 18.7%; $P=.003$) and macrosomia (13.5% vs 10.9%; $P=.043$) compared with those conceived after the natural ovulation regimen.

Subgroup analyses according to age, BMI, endometrial thickness on the day of hCG administration during ovarian stimulation, and developmental stage of transferred embryo were performed in terms of pregnancy outcomes (Figure 2). No significant interaction was found between endometrial preparation regimens and any of the subgroups. When stratified by age, we observed that the greater likelihood of achieving a healthy live birth after the natural ovulation regimen vs programmed regimen remained within both age subgroups. Compared with the programmed regimen, the natural ovulation regimen was associated with 22.2% lower probability of clinical pregnancy loss among women aged <35 years (relative risk [RR], 0.778; 95% confidence interval [CI], 0.647−0.936), whereas the between-group difference did not reach statistical significance among women aged ≥35 years (RR, 0.838; 95% CI, 0.667−1.051). When stratified by BMI, women using the natural ovulation regimen for endometrial preparation were 15.7% more likely to achieve a healthy live birth in the subgroup of BMI <28 kg/m^2, and 43.5% less likely to experience clinical pregnancy loss (RR, 0.565; 95% CI, 0.388−0.821) in the subgroup of BMI ≥28 kg/m^2 compared with women using the programmed regimen. When stratified by embryo stage at transfer, the statistically significant between-group difference in pregnancy outcomes was observed within both subgroups. When stratified by endometrial thickness on hCG trigger day, the statistically significant difference in pregnancy outcomes between the 2 treatment groups was only present in the subgroup of endometrial thickness ≥8 mm, but not in the subgroup of endometrial thickness <8 mm. We further performed stratified analysis by endometrial thickness before FET and also found that the results were consistent only in the subgroup of endometrial thickness ≥8 mm (Supplementary Table II).

Comment

Principal findings

In this study, after controlling for confounding factors by PS matching, we found that the natural ovulation regimen for endometrial preparation before FET was associated with a higher likelihood of achieving a healthy live birth compared with the programmed regimen. In addition, women with the natural ovulation regimen were less likely to experience clinical pregnancy loss and HDP, and less likely to deliver LGA singletons when compared with women with the programmed regimen.

Strengths and limitations

The strength of this study was that we reported the difference in the healthy live birth rate between the programmed and natural ovulation regimen, with a PS matching approach. In addition, the study had a sufficient sample size to provide adequate statistical power. To minimize the possibility of selection bias and confounders, only women undergoing their first FET cycles were enrolled in our study, and PS matching was used to achieve well-balanced variables across groups. However, certain limitations of this study should be considered for the interpretation of results. Despite effective PS matching for both preconception and IVF-related variables, this study was inherently limited by the retrospective collection of data, with a possibility of selection bias and bias by residual confounding or unmeasured confounders. Secondly, this was a single-center study of the Chinese Han population, limiting the generalizability of our findings. Thirdly, ovulation can also be triggered by hCG injection to facilitate FET scheduling, a strategy referred to as the "modified natural regimen." Whether our findings also apply to the modified natural regimen remains to be evaluated. Fourth, this study did not address the effects of the type, dosage, and route of hormonal agents for luteal support on clinical outcomes, which still remain inconclusive. Finally, only ovulatory women with regular menstrual cycles were included in this study; further studies comparing the programmed regimen with the

TABLE 1
Comparisons of baseline characteristics before and after propensity score matching

Characteristics	Before matching			After matching		
	Natural ovulation regimen (N=4690)	Programmed regimen (N=3134)	*P* value	Natural ovulation regimen (N=2861)	Programmed regimen (N=2861)	*P* value
Age (y)	32.14±4.58	31.50±4.81	<.0001	31.74±4.50	31.62±4.83	.316
Age ≥35, n (%)	1385 (29.5)	819 (26.1)	.001	762 (26.6)	771 (26.9)	.788
BMI (kg/m^2)	23.22±3.30	23.85±3.59	<.0001	23.70±3.44	23.69±3.53	.959
BMI ≥28, n (%)	413 (8.8)	407 (13.0)	<.0001	319 (11.1)	343 (12.0)	.321
Duration of infertility (y)	3 (1.5–5)	3 (1.5–5)	.359	3 (2.0–5.0)	3 (1.5–5.0)	.744
Nulligravida, n (%)	2618 (55.8)	1707 (54.5)	.238	1573 (55.0)	1567 (54.8)	.873
Nulliparity, n (%)	1580 (33.7)	896 (28.6)	<.0001	863 (30.2)	853 (29.8)	.773
Number of previous miscarriages, n (%)			.232			.415
0	2617 (55.8)	1731 (55.2)		1576 (55.1)	1583 (55.3)	
1–2	1656 (35.3)	1152 (36.8)		1037 (36.2)	1057 (36.9)	
≥3	417 (8.9)	251 (8.0)		248 (8.7)	221 (7.7)	
History of uterine adhesion, n (%)	201 (4.3)	217 (6.9)	<.0001	169 (5.9)	158 (5.5)	.531
Causes of infertility, n (%)			.016			.166
Tubal factors	3545 (75.6)	2437 (77.8)		2192 (76.6)	2222 (77.7)	
Male factors	914 (19.5)	531 (16.9)		536 (18.7)	488 (17.1)	
Others	231 (4.9)	166 (5.3)		133 (4.6)	151 (5.3)	
FSH (IU/L)	6.81±2.13	6.72±2.44	.105	6.74±2.17	6.75±2.44	.802
Total testosterone (ng/dL)	23.79±13.38	26.03±13.82	<.0001	25.04±14.04	25.26±13.23	.525
AMH (ng/mL)	2.86 (1.72–4.57)	3.26 (1.83–5.52)	<.0001	3.17 (1.77–5.11)	3.12 (1.76–5.26)	.843
AFC	13.94±5.89	15.23±7.32	<.0001	14.62±6.18	14.62±6.54	.985
FBG (mmol/L)	5.22±0.45	5.24±0.47	.124	5.24±0.47	5.23±0.47	.583
Protocols for COH, n (%)						
GnRH-agonist long	2258 (48.1)	1283 (40.9)	<.0001	1232 (43.1)	1233 (43.1)	.112
GnRH-antagonist	1085 (23.1)	870 (27.8)		716 (25.0)	756 (26.4)	
GnRH-agonist short	997 (21.3)	602 (19.2)		634 (22.2)	567 (19.8)	
Others	350 (7.5)	379 (12.1)		279 (9.8)	305 (10.7)	
Total gonadotropin dose (IU)	1685 (1350–2275)	1800 (1350–2475)	<.0001	1725 (1350–2400)	1800 (1350–2400)	.781
Estradiol level on hCG trigger day (pg/mL)	3416 (2203–5256)	3355 (2095–5369)	.253	3390 (2177 –5228)	3364 (2108–5392)	.887
EMT on hCG trigger day (mm)	10.67±2.03	10.49±2.18	<.0001	10.52±2.08	10.56±2.13	.480
EMT on hCG trigger day <8 mm, n (%)	258 (5.5)	287 (9.2)	<.0001	221 (7.7)	212 (7.4)	.653
Number of retrieved oocytes	12.38±6.11	12.51±6.81	.413	12.38±6.14	12.43±6.79	.761
Fertilization method, n (%)			.897			.977
IVF	3348 (71.4)	2233 (71.3)		2044 (71.4)	2043 (71.4)	
ICSI	1342 (28.6)	901 (28.7)		817 (28.6)	818 (28.6)	
Embryo developmental stage at transfer, n (%)			.997			.790
D5	3553 (75.8)	2373 (75.7)		2154 (75.3)	2176 (76.1)	
D6	1078 (23.0)	721 (23.0)		670 (23.4)	650 (22.7)	
D7	59 (1.3)	40 (1.3)		37 (1.3)	35 (1.2)	

Data are presented as mean±standard deviation or median (interquartile range) for continuous variables and number (percentage) for categorical variables.

AFC, antral follicle count; *AMH*, anti-Müllerian hormone; *BMI*, body mass index; *COH*, controlled ovarian hyperstimulation; *EMT*, endometrial thickness; *FBG*, fasting blood glucose; *FSH*, follicle-stimulating hormone; *GnRH*, gonadotropin-releasing hormone; *hCG*, human chorionic gonadotropin; *ICSI*, intracytoplasmic sperm injection; *IVF*, in vitro fertilization.

Wang. Healthy live birth outcome after frozen embryo transfer prepared by natural ovulation vs programmed regimen. Am J Obstet Gynecol Glob Rep 2023.

TABLE 2
Pregnancy outcomes and neonatal outcomes between the 2 groups after propensity score matching

Characteristics	Natural ovulation regimen (N=2861)	Programmed regimen (N=2861)	*P* value
Conception, n (%)	1948 (68.1)	1913 (66.9)	.323
Clinical pregnancy, n (%)	1724 (60.3)	1657 (57.9)	.072
Clinical pregnancy loss, n (%)	276/1724 (16.0)	327/1657 (19.7)	.005
Live birth, n (%)	1448 (50.6)	1330 (46.5)	.002
Singleton live birth	1418 (49.6)	1307 (45.7)	.003
Twin live birth	30 (1.0)	23 (0.8)	.334
Hypertensive disorder complicating pregnancy, n (%)	67/1724 (3.9)	100/1657 (6.0)	.004
GDM, n (%)	148/1724 (8.6)	123/1657 (7.4)	.214
Preterm birth in singletons, n (%)	98/1418 (6.9)	98/1307 (7.5)	.554
Birthweight in singletons, (g)	3394.96±503.87	3441.50±539.97	.020
LGA in singletons, n (%)	265/1418 (18.7)	305/1307 (23.3)	.003
SGA in singletons, n (%)	47/1418 (3.3)	46/1307 (3.5)	.768
Macrosomia in singletons, n (%)	155/1418 (10.9)	176/1307 (13.5)	.043
Low birthweight in singletons, n (%)	56/1418 (3.9)	48/1307 (3.7)	.706
Healthy live birth, n (%)	1023 (35.8)	875 (30.6)	<.0001

Data are presented as mean±standard deviation for continuous variables and number percentage for categorical variables.

GDM, gestational diabetes mellitus; *LGA*, large for gestational age; *SGA*, small for gestational age.

Wang. Healthy live birth outcome after frozen embryo transfer prepared by natural ovulation vs programmed regimen. Am J Obstet Gynecol Glob Rep 2023.

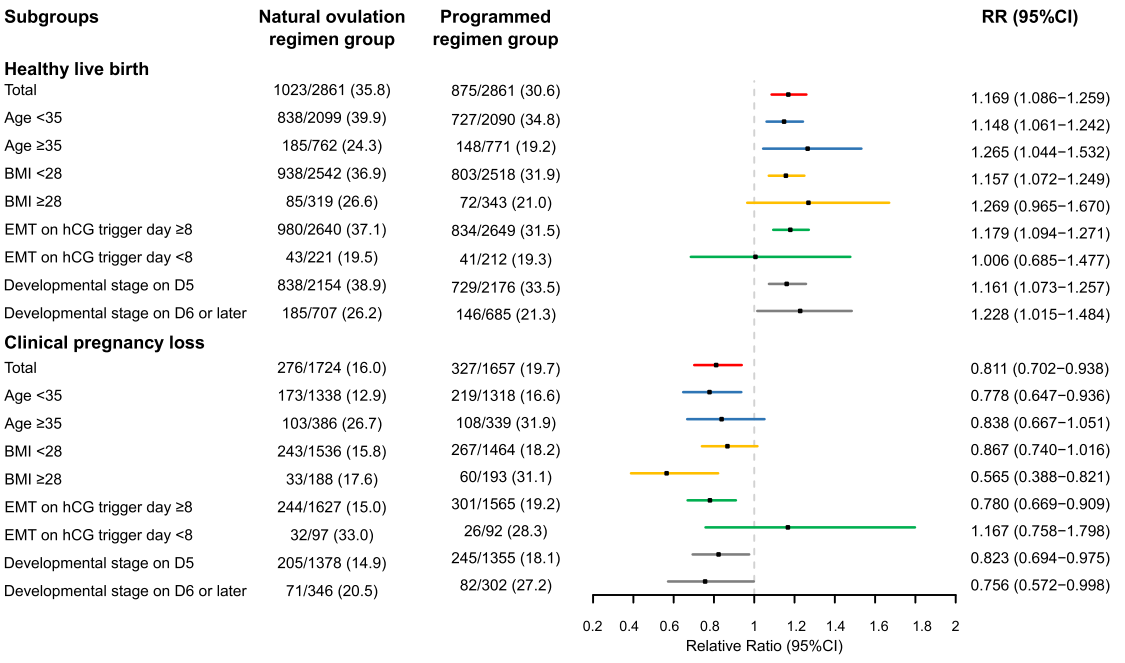
stimulated regimen for anovulatory women, such as those with polycystic ovary syndrome, are needed.

Clinical implications

With technological advances, the subject of maternal and neonatal health after IVF treatment has attracted more focus. A composite measure of "healthy live birth rate" may be more reflective of IVF success than the currently used outcome measure—the live birth rate. In this study, we observed that women with the natural ovulation regimen were 16.9% more likely to achieve a healthy live birth compared with women with the programmed regimen. We believe that the 5.2% absolute difference in the healthy live birth rate was not only statistically significant but also clinically meaningful. Further analyses did not show that the association was related to age, BMI, previous endometrial thickness, or embryo stage at transfer, although our sample size may have been underpowered to detect a statistically significant interaction.

Our study showed that the natural ovulation regimen was associated with a higher rate of singleton live birth and a lower risk of clinical pregnancy loss compared with the programmed regimen. Previous studies comparing the natural ovulation regimen with the programmed regimen have reported conflicting results in terms of live birth rate. A 2020 Cochrane review[6] (4 randomized controlled trials; 1285 participants) suggested that there was very low-quality evidence in favor of one regimen over the other. The largest study by Groenewoud et al,[15] with a sample size of 734 women, indicated that the live birth rate after the natural ovulation regimen was comparable with that of the programmed regimen (14.5% vs 12.1%). Notably, the live birth rate was remarkably low, which may have been due to >90% of women having undergone single cleavage-stage embryo transfer. Moreover, the planned sample size of 1150 patients required for adequate statistical power was not reached. Two retrospective studies including euploid blastocyst transfer also failed to detect a difference in the live birth rate between the natural and programmed regimen among women with regular menstrual cycles.[16,17] Our results were consistent with several studies suggesting that the natural ovulation regimen may be superior to the programmed regimen in terms of pregnancy outcomes.[18–20] A recent retrospective study by Godiwala et al[18] found that women with the programmed regimen had a significantly higher probability of clinical pregnancy loss than women with the natural ovulation regimen (17.2% vs 10.1%), and the association remained statistically significant after controlling for potential confounders (adjusted RR, 0.62; 95% CI, 0.46–0.84). Likewise, Liu et al reported a higher risk of clinical pregnancy loss and a lower likelihood of live birth after the programmed regimen relative to the

FIGURE 2
Subgroup analyses in terms of pregnancy outcomes



RRs (95% CIs) for healthy live birth and clinical pregnancy loss in the overall population, stratified by age, BMI, EMT on the day of hCG trigger, and the developmental stage of transferred embryo, respectively.

BMI, body mass index; *CI*, confidence interval; *EMT*, endometrial thickness; *hCG*, human chorionic gonadotropin; *RR*, risk ratio.

Wang. Healthy live birth outcome after frozen embryo transfer prepared by natural ovulation vs programmed regimen. Am J Obstet Gynecol Glob Rep 2023.

natural ovulation regimen among women aged 20 to 35 years.[19] In a post hoc secondary analysis of a trial comparing the live birth rate following fresh vs frozen single blastocyst transfer, the programmed regimen was found to be significantly associated with a higher risk of biochemical miscarriage and a lower live birth rate compared with the natural ovulation regimen.[20]

Our findings also indicated increased risk of hypertensive disorders during pregnancy after the programmed regimen compared with the natural ovulation regimen, which has been highlighted by multiple observational studies.[8,21−24] Increasing evidence has emerged supporting that the absence of a CL, at least partially, predisposes women to clinical pregnancy loss and the development of preeclampsia after the programmed regimen.[25,26] The CL serves as the major source of progesterone that is indispensable in early pregnancy. As mentioned previously, luteal support in the programmed regimen entails exogenous administration of high-dose steroid hormone because of an absence of CL resulting from the suppression of ovulation. However, in addition to estrogen and progesterone, other secretory products generated by the CL, such as relaxin, may also be implicated in optimizing implantation, placentation, and maternal vascular health. In vivo studies in animal models have supported this hypothesis, suggesting that relaxin may play a pivotal role in decidualization, endometrial immune tolerance, endometrial vascular remodeling, uterine artery adaptation, endothelial vasodilator function, smooth muscle reactivity, and placental perfusion.[27−29] Conversely, the protocol for luteal phase support used in programmed FET cycles is also essential for pregnancy establishment and pregnancy maintenance. There is still no consensus on the optimal dosage, route, and duration of estrogen and progesterone administration for the programmed regimen.[6] The possible effect of suboptimal corpus luteal support with estrogen and progesterone for programmed regimen on the increased risk of clinical pregnancy loss and on the development of preeclampsia has been reconsidered.[26] Moreover, it has been hypothesized that suboptimal hormonal levels, or even "normal" hormonal levels that may be still relatively inadequate in a subset of women, may interfere with optimal decidualization and placentation through immune response alteration.[30] However, how to define the suboptimal hormonal levels and how to identify the subset of population who may need enhanced corpus luteal support remains unanswered.

Our findings were consistent with the results from a meta-analysis of observational data revealing that assisted reproductive technology−conceived infants born after the programmed regimen have greater birthweight (mean difference, 47.38 g; P=.04) and higher risk of LGA (odds ratio, 1.10; 95% CI, 1.02−1.19) than those born after the natural ovulation regimen.[9] However, the pathophysiological mechanisms responsible for fetal weight abnormality resulting

from the programmed regimen remain unclear. Nevertheless, the possible correlation might be attributed to the vital role of the absence of a CL or suboptimal hormonal levels in causing epigenetic alterations and alterations in the uterine microenvironment, thereby influencing placentation and fetal growth.[31,32]

Conclusion

Our research suggested that the natural ovulation regimen for endometrial preparation before FET may be superior to the programmed regimen with regard to higher likelihood of a healthy live birth and lower risk of pregnancy loss and maternal HDP. Further confirmation from prospective studies is warranted. ■

ACKNOWLEDGMENTS

The authors thank the clinicians, nurses, laboratory staff, and information engineers of the Center for Reproductive Medicine Affiliated to Shandong University for their contribution.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.xagr.2023.100210.

REFERENCES

1. Shi Y, Sun Y, Hao C, et al. Transfer of fresh versus frozen embryos in ovulatory women. N Engl J Med 2018;378:126–36.
2. Wei D, Liu JY, Sun Y, et al. Frozen versus fresh single blastocyst transfer in ovulatory women: a multicentre, randomised controlled trial. Lancet 2019;393:1310–8.
3. Chen ZJ, Shi Y, Sun Y, et al. Fresh versus frozen embryos for infertility in the polycystic ovary syndrome. N Engl J Med 2016;375:523–33.
4. Roque M, Haahr T, Geber S, Esteves SC, Humaidan P. Fresh versus elective frozen embryo transfer in IVF/ICSI cycles: a systematic review and meta-analysis of reproductive outcomes. Hum Reprod Update 2019;25:2–14.
5. Berntsen S, Pinborg A. Large for gestational age and macrosomia in singletons born after frozen/thawed embryo transfer (FET) in assisted reproductive technology (ART). Birth Defects Res 2018;110:630–43.
6. Glujovsky D, Pesce R, Sueldo C, Quinteiro Retamar AM, Hart RJ, Ciapponi A. Endometrial preparation for women undergoing embryo transfer with frozen embryos or embryos derived from donor oocytes. Cochrane Database Syst Rev 2020;10:CD006359.
7. Ghobara T, Gelbaya TA, Ayeleke RO. Cycle regimens for frozen-thawed embryo transfer. Cochrane Database Syst Rev 2017;7:CD003414.
8. Ginström Ernstad E, Wennerholm UB, Khatibi A, Petzold M, Bergh C. Neonatal and maternal outcome after frozen embryo transfer: increased risks in programmed cycles. Am J Obstet Gynecol 2019;221:126.e1–18.
9. Rosalik K, Carson S, Pilgrim J, et al. Effects of different frozen embryo transfer regimens on abnormalities of fetal weight: a systematic review and meta-analysis. Hum Reprod Update 2021;28:1–14.
10. Maheshwari A, Bell JL, Bhide P, et al. Elective freezing of embryos versus fresh embryo transfer in IVF: a multicentre randomized controlled trial in the UK (E-Freeze). Hum Reprod 2022;37:476–87.
11. Zhu Z, Yuan L, Wang J, et al. Mortality and morbidity of infants born extremely preterm at tertiary medical centers in China from 2010 to 2019. JAMA Netw Open 2021;4:e219382.
12. Sacchi C, Marino C, Nosarti C, Vieno A, Visentin S, Simonelli A. Association of intrauterine growth restriction and small for gestational age status with childhood cognitive outcomes: a systematic review and meta-analysis. JAMA Pediatr 2020;174:772–81.
13. Doty MS, Chen HY, Sibai BM, Chauhan SP. Maternal and neonatal morbidity associated with early term delivery of large-for-gestational-age but nonmacrosomic neonates. Obstet Gynecol 2019;133:1160–6.
14. Dai L, Deng C, Li Y, et al. Birth weight reference percentiles for Chinese. PLoS One 2014;9:e104779.
15. Groenewoud ER, Cohlen BJ, Al-Oraiby A, et al. A randomized controlled, non-inferiority trial of modified natural versus artificial cycle for cryo-thawed embryo transfer. Hum Reprod 2016;31:1483–92.
16. Jin Z, Shi H, Bu Z, et al. Live birth rates after natural cycle versus hormone replacement therapy for single euploid blastocyst transfers: a retrospective cohort study. Reprod Biomed Online 2021;43:1002–10.
17. Fu Y, Chen D, Cai B, et al. Comparison of two mainstream endometrial preparation regimens in vitrified-warmed embryo transfers after PGT. Reprod Biomed Online 2022;44:239–46.
18. Godiwala P, Makhijani R, Bartolucci A, et al. Pregnancy outcomes after frozen-thawed embryo transfer using letrozole ovulation induction, natural, or programmed cycles. Fertil Steril 2022;118:690–8.
19. Liu X, Shi W, Shi J. Natural cycle frozen-thawed embryo transfer in young women with regular menstrual cycles increases the live-birth rates compared with hormone replacement treatment: a retrospective cohort study. Fertil Steril 2020;113:811–7.
20. Lin J, Zhao J, Hao G, et al. Maternal and neonatal complications after natural vs. hormone replacement therapy cycle regimen for frozen single blastocyst transfer. Front Med (Lausanne) 2020;7:338.
21. Wang Z, Liu H, Song H, et al. Increased risk of pre-eclampsia after frozen-thawed embryo transfer in programming cycles. Front Med (Lausanne) 2020;7:104.
22. von Versen-Höynck F, Schaub AM, Chi YY, et al. Increased preeclampsia risk and reduced aortic compliance with in vitro fertilization cycles in the absence of a corpus luteum. Hypertension 2019;73:640–9.
23. Asserhøj LL, Spangmose AL, Aaris Henningsen AK, et al. Adverse obstetric and perinatal outcomes in 1,136 singleton pregnancies conceived after programmed frozen embryo transfer (FET) compared with natural cycle FET. Fertil Steril 2021;115:947–56.
24. von Versen-Höynck F, Narasimhan P, Selamet Tierney ES, et al. Absent or excessive corpus luteum number is associated with altered maternal vascular health in early pregnancy. Hypertension 2019;73:680–90.
25. Pereira MM, Mainigi M, Strauss JF. Secretory products of the corpus luteum and preeclampsia. Hum Reprod Update 2021;27:651–72.
26. Conrad KP, von Versen-Höynck F, Baker VL. Potential role of the corpus luteum in maternal cardiovascular adaptation to pregnancy and preeclampsia risk. Am J Obstet Gynecol 2022;226:683–99.
27. Einspanier A, Lieder K, Husen B, et al. Relaxin supports implantation and early pregnancy in the marmoset monkey. Ann N Y Acad Sci 2009;1160:140–6.
28. Goldsmith LT, Weiss G, Palejwala S, et al. Relaxin regulation of endometrial structure and function in the rhesus monkey. Proc Natl Acad Sci U S A 2004;101:4685–9.
29. Hayes ES, Curnow EC, Trounson AO, Danielson LA, Unemori EN. Implantation and pregnancy following in vitro fertilization and the effect of recombinant human relaxin administration in Macaca fascicularis. Biol Reprod 2004;71:1591–7.
30. Al-Lami RA. Immune-altering roles of progesterone and subsequent development of preeclampsia in artificial cycles of frozen embryo transfer. Am J Obstet Gynecol 2022;227:675–6.
31. Dwi Putra SE, Reichetzeder C, Hasan AA, et al. Being born large for gestational age is associated with increased global placental DNA methylation. Sci Rep 2020;10:927.
32. Dumolt JH, Powell TL, Jansson T. Placental function and the development of fetal overgrowth and fetal growth restriction. Obstet Gynecol Clin North Am 2021;48:247–66.