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ASSISTED REPRODUCTION TECHNOLOGIES

Increased pregnancy complications following frozen-thawed embryo transfer during an artificial cycle

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Abstract

Purpose This study aimed to clarify the risks of adverse pregnancy outcomes in patients and their offspring after frozen embryo transfer (FET) during an artificial cycle (AC).

Methods We conducted a retrospective cohort study that included all FET cycles and subsequent deliveries in a single centre between August 2013 and March 2016. Pregnancy, obstetric and neonatal outcomes were compared among patients treated during an AC or a natural cycle with luteal phase support (NC-LPS). Multivariate logistic regression was performed to evaluate the relationship between endometrial preparation schemes and pregnancy, obstetric and neonatal outcomes.

Results AC-FET was not a significant risk factor for clinical pregnancy rate, multiple birth rate or miscarriage rate after adjusting for potential confounders. However, AC-FET was a significant risk factor for ectopic pregnancy rate (adjusted odds ratio (AOR), 1.738; 95% confidence interval (CI), 1.086–2.781) and live birth rate (AOR, 0.709; 95% CI, 0.626–0.802). Regarding obstetric outcomes, AC-FET was found to be associated with an increased risk for hypertension disorder (AOR, 1.780; 95% CI, 1.262–2.510) and caesarean section (AOR, 1.507; 95% CI, 1.195–1.900). In multiples, birth weight (2550 g (2150–2900 g) in AC-FET vs. 2600 g (2350–2900 g) in NC-LPS; $P = 0.023$), gestational age (36.6 weeks (35.3–37.6 weeks) vs. 37.1 weeks (36.1–37.9 weeks); $P < 0.001$), and z-score (− 0.5 (− 1.1, − 0.0) vs. − 0.4 (− 1.0, 0.2); $P = 0.009$) were higher in the NC-LPS group than in the AC-FET group, although there were no differences in these variables among singletons.

Conclusion Compared with NC-LPS, AC-FET seemed to have a negative effect on obstetric outcomes.

Keywords Natural cycle · Artificial cycle · Pregnancy · Obstetric · Neonatal outcomes

Introduction

Thus far, numerous studies have followed children born after frozen embryo transfer (FET) [1–4]. The Society for Assisted Reproductive Technology reported that FET cycles are now more successful than fresh embryo transfer (ET) cycles [5], and an increasing number of studies have claimed superior pregnancy outcomes with FET cycles compared to fresh ET

S. J., X.L. should be considered similar in author order.

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[6–9], such as an increased clinical pregnancy rate (CPR) among patients with polycystic ovary syndrome (PCOS) [10]. Since FET reduces the risk of hyperstimulation and avoids injury to the female caused by repeated oocyte recovery and embryo waste, frozen embryos have been widely accepted and utilised in various reproductive centres.

However, compared with fresh ET, FET is thought to be associated with several adverse perinatal outcomes, such as increased incidences of pregnancy-induced hypertension [11–13], abnormal placenta formation [12, 14], post-term birth [15], and macrosomia [2, 16, 17]. The underlying mechanism is not yet clear. Endometrial damage during ET and epigenetic changes caused by cryopreservation and embryo culture in vitro may be the causes of these phenomena [14, 15, 18, 19].

Endometrium preparation schemes designed for the thawed embryo generally include natural cycles (NC-FET) and artificial cycles (AC-FET), in which the endometrium is artificially prepared with exogenous steroid hormone therapy. For NC-FET, the moment of ovulation can be estimated based on the detection of the luteinizing hormone (LH) surge in either urine or blood (constituting 'true' NC-FET) or after triggering ovulation of the dominant follicle using human chorionic gonadotrophin (hCG) ('modified' NC-FET) [20] or supported the luteal phase with progesterone ('natural cycle with luteal phase support (NC-LPS)) [21]. An AC helps patients with irregular cycles reduce the frequency of hospital visits, easily schedule the date of FET, and decrease ET cancellation rates [22, 23]. For these reasons, many eumenorrheic women undergo AC-FET. Numerous studies have tried to identify the optimal scheme to obtain better pregnancy outcomes and to avoid or reduce adverse obstetric and neonatal outcomes [22, 24]. However, which is the best scheme sitll unclear. In fact, the supraphysiological dose of hormones utilised in assisted reproductive technology (ART) procedures especially steroid hormone concentrations in the first trimester (oestradiol (E2), prolactin, progesterone (P)), and patient fertility-related problems may influence pregnancy, obstetric and neonatal outcomes [25–28]. Therefore, we aimed to determine whether AC-FET increases the risk of adverse pregnancy, obstetric and neonatal outcomes.

Materials and methods

Patient population

The study population comprised patients from Citic-Xiangya Hospital who underwent FET after an unsuccessful fresh ET, in which the protocol stipulated a gonadotropin-releasing hormone (GnRH) agonist or follicle-stimulating hormone (FSH) or other protocol. Indications for the previous ART cycle were either three unsuccessful intrauterine inseminations, tubal factor (IVF) or male factor (ICSI). The GnRH agonist and FSH protocol was mainly performed.

Inclusion criteria were women undergoing FET during a NC-LPS or women undergoing FET during an AC with E2 in the follicular phase. The included women had at least one blastocyst or two cleavage-stage embryos in storage, regular ovulatory cycles, and at most two previous ET cycles. Exclusion criteria were stimulation protocols with either clomiphene citrate (CC), gonadotrophins, GnRH agonist (GnRHa), or GnRH antagonist (GnRHant) and the administration of hCG in the luteal phase. We excluded patients who can only do artificial cycles such as suffering from ovulation disorders, Asherman syndrome, Polycystic Ovary Syndrome, uterine malformation and so on.

Study design

The study protocol was approved by the Ethics Committee of Citic-Xiangya Hospital (no. 31171379). Our study occurred between August 2013 and March 2016 and included 6201 newborns from 11,037 FET cycles (Fig. 1).

Procedure

The decision to proceed with a NC or an AC was reached through a combination of patient preference and physician guidance. Some patients may prefer to ACs which are easier to monitor.

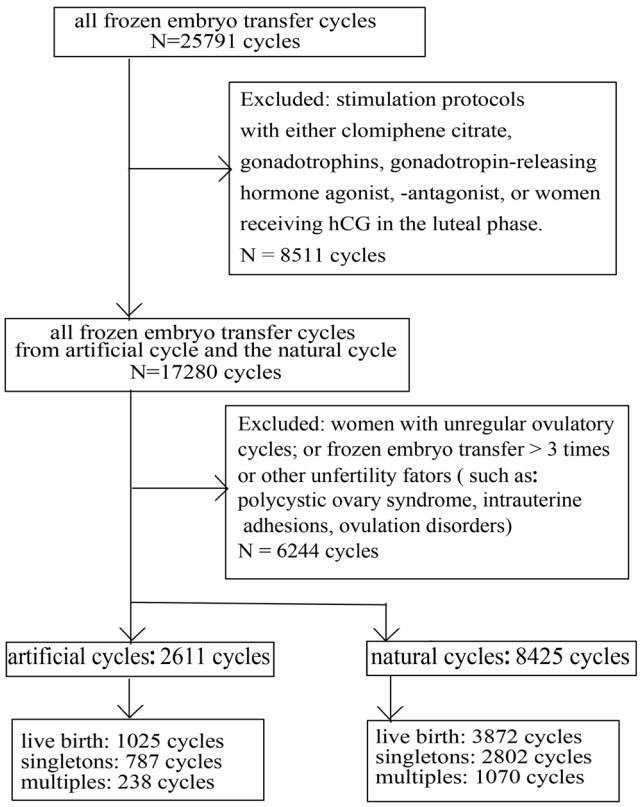

Fig. 1 Flow diagram showing the distribution of the study populations

NC Women with an NC did not take any medication during the follicular phase. Transvaginal sonography was performed on day ten. Subsequently, ultrasounds were performed until the endometrium thickness was at least 8 mm and the main follicle reached 18–22 mm. Ovulation predictor kits were also used for to monitor ovulation. When ovulation occurred, FET was scheduled based on the embryo stage at freezing (i.e. day 3 for cleavage embryos and day 5 for blastocysts). The luteal phase was supported with progesterone (600 mg/day, 3 × 200 mg; Duphaston, Abbott Biologicals B.V., The Netherlands) applied vaginally 2 days before FET.

AC Women undergoing an AC started on the first day of their natural menstrual cycle. Oestrogen (Progynova, DELPHARM Lille S.A.S., France) (2 mg oestradiol valerate) was administered orally: one pill on days 1, 2, 3 and 4; two pills on days 5, 6 and 7; three pills on days 8, 9, 10 and 11; four pills on days 12, 13, 14, 15 and 16; and two pills on days 17 to 31. When the endometrial thickness reached at least 8 mm, dydrogesterone was administered orally (10 mg per 12 h; Duphaston, Abbott Biologicals B.V., The Netherlands), and progesterone was administered vaginally (200 mg, three times a day; Utrogestan, Capsugel, France) for luteal phase support.

All women were screened for endometrial responsiveness using transvaginal sonography, and blood samples were taken to measure luteinizing hormone (LH), E2 and P levels before FET in both groups. After ET, blood samples were taken to measure β-hCG levels after 12 days for cleavage embryos and 10 days for blastocysts. If β-hCG levels were higher than usual, further hospital visits were scheduled 28 days later to confirm clinical pregnancy and singleton or twin pregnancy. Embryos were frozen by vitrification according to the procedure described previously [29]. More than 99% of the women in our study population were of Han ethnicity. Smoking and alcohol use was very uncommon in this population. Due to the one-child policy, 90% of the population was primipara (data not shown).

Hypertensive disorder was defined as sustained (on at least two occasions 6 h apart) blood pressure ≥ 140/90 mmHg after 20 weeks, with or without proteinuria and other signs or symptoms of preeclampsia and without a prior history of hypertension. Because the diagnostic criteria for preeclampsia and gestational hypertension varied across the study period and between the countries [30], we considered hypertensive disorders in pregnancy as a single, combined outcome (International Classification of Diseases, Tenth Revision, ICD-10: O11, O13-O16). Small for gestational age (SGA) was defined as a birth weight based on gestational week below the 10th percentile, and large for gestational age (LGA) was defined similarly above the 90th percentile [31, 32]. Stillbirths (intrauterine or intrapartum death of a child born at a gestational age of ≥ 20 weeks or weighing ≥ 500 g), early neonatal deaths (death of a live born infant before day 7) and perinatal deaths (number of stillborn infants and early neonatal deaths) were recorded [8]. The number of late neonatal deaths (death of a live born infant between days 7 and 28) and infant deaths (death of a live born infant after day 28) were also reported for information only; please refer to Belva's article [8].

Statistical analysis

Data were statistically analysed using SPSS 22.0. Non-normally distributed data are presented as the median (1st quartile–3rd quartile), and the Mann-Whitney test was used for comparisons. Categorical variables are presented as percentages, and the χ^2 test or Fisher's exact test was used for comparisons. The z-score was used to evaluate the associations with birth weight and embryo culture duration after adjusting for infant gender and gestational age: z-score = (newborn birth weight—mean birth weight at the same gestational age for the same gender in the reference population)/ standard deviation (SD) in the same reference population. The reference population was derived from the latest publication regarding gestational age-specific birth weights of Chinese singletons or multiples [31, 32]. Multivariate logistic regression analysis was used to adjust for potential confounding factors between endometrium preparation schemes and pregnancy, obstetric, and neonatal adverse outcomes. Confounding factors were selected based on their clinical relevance and previous knowledge, and they were unevenly distributed between both groups of women studied. $P < 0.05$ was considered statistically significant.

Results

Demographic data

The research group and hospital information engineer extracted data from the hospital electronic medical records according to criteria set forth on the standardised data collection form. Discrete data (i.e. data that were not stored as free text) were pulled directly from the medical records. The following information was collected: maternal demographic characteristics, medical history, treatment programmes, reproductive and prenatal history, labour and delivery summaries, and postpartum and neonatal information.

Pregnancy outcomes

Demographic and clinical data, including maternal age, BMI, basal FSH, endometrial thickness, blastomere/blastocyst stage, the day of development of frozen embryos and reasons for infertility, shown in Table 1. BMI, basal FSH, endometrial thickness, blastomere/blastocyst stage and endometrium thickness were significantly different between the two groups.

Table 1 Demography and treatment results

	AC-FET	NC-LPS	*P* value
No. of cycles	2611	8425	
Maternal age (year)	31 (28, 35)	31 (28, 35)	0.722
BMI (kg/m^2)	21.6(19.9, 23.5)	21.2 (19.6, 23.1)	< 0.001
Basal FSH (miu/ml)	6.11 (5.1, 7.4)	6.2 (5.3, 7.4)	0.005
Primary subfertility	1050 (40.2)	3500 (41.5)	0.228
Causes of infertility (%)			
Teratospermia	344 (13.2)	1113 (13.2)	0.960
Oligoasthenozoospermia	225 (8.6)	793 (9.4)	0.220
Oviduct factor	2345 (89.8)	7524 (89.3)	0.462
Endometrial thickness (mm) on translate day	10.8 (9.9, 11.7)	11.4 (10.4, 12.5)	< 0.001
Number of transferred embryos (%)			0.605
1	428 (16.4)	1313 (15.6)	
2	1997 (76.5)	6514 (77.3)	
3	186 (7.1)	598 (7.1)	
Blastomere or blastocyst transfer (%)			0.015
Blastomere transfer	1605 (61.5)	5399 (64.1)	
Blastocyst transfer	1006 (38.6)	3026 (35.9)	
The day of development of frozen embryos			0.115
Day 3	1605 (61.5)	5396 (64.0)	
Day 4	1 (0.0)	3 (0.0)	
Day 5	783 (30.0)	2379 (28.2)	
Day 6	222 (8.5)	647 (7.7)	
Good-quality rate of the embryo implanted	1446 (56.8)	4703 (55.4)	0.188
Clinical outcomes			
Clinical pregnancy rate	1326 (50.8)	4708 (55.9)	< 0.001
Miscarriage rate	253 (19.1)	748 (15.9)	0.006
Ectopic pregnancy rate	44 (3.3)	75 (1.6)	0.006
Live birth rate	1025 (39.3)	3872 (46.0)	< 0.001
Multiple rate[d]	235 (22.9)	1063 (27.5)	< 0.001

Continuous measurements are summarised as median (1st quartile–3rd quartile) if asymmetrically distributed. Nominal measurements are summarised as *n* (%) unless stated otherwise. AC-FET, artificial cycle; NC-LPS, natural cycles with luteal phase support; BMI, body mass index; FSH, follicle-stimulating hormone; ET, embryo transfer

The CPR, live birth rate (LBR), and multiples rate were 55.9, 46.0 and 27.5% in the NC-LPS group and 50.8, 39.3 and 22.9% in the AC-FET group, respectively; these values were significantly higher in the NC-LPS group than in the AC-FET group ($P < 0.05$). The miscarriage rate and ectopic pregnancy rate were higher in the AC-FET group (19.1 vs. 1.6% and 15.9 vs. 3.3% in the NC-LPS group, respectively; $P < 0.05$).

Obstetric outcomes

As shown in Table 2, BMI, basal FSH, endometrial thickness, blastomere/blastocyst stage, the day of development of frozen embryos and endometrium thickness were significantly different between the two groups. The percentages of patients with hypertension disorder (4.2 in NC-LPS vs. 7.2% in AC-FET; $P < 0.001$) and who underwent caesarean section (78.4 vs. 85.9%; $P < 0.001$) were higher in after AC-FET than after NC-LPS. There were no differences in the incidence of other obstetric complications.

Neonatal outcomes

The rates of neonatal death, SGA and LGA did not differ between the two groups among singletons or multiples, as shown in Table 3.

Singleton outcomes

Stillbirths were more common after AC-FET than NC-LPS (0.6 vs. 0.1%, $P = 0.029$). Newborn birth weight (3400 g (3105 g–3700 g) in NC-LPS vs. 3400 g (3100–3700 g) in AC-FET; $P = 0.299$), gestational age (39.1 weeks (38.4–

Table 2 Maternal and obstetric parameters in pregnancies with live born after AC-FET group and NC-LPS group

	AC-FET	NC-LPS	*P* value
No. of cycles	1025	3872	
Maternal age (year)	30 (27, 33)	30 (27, 33)	0.506
BMI (kg/m^2)	21.3 (19.7, 23.2)	21.1 (19.5, 22.9)	0.012
Basal FSH (miu/ml)	5.9 (5.0, 7.1)	6.2 (6.1, 7.2)	0.003
Primary subfertility	452 (44.1)	1717 (44.3)	0.888
Endometrial thickness (mm) on translate day	10.9 (10.0, 11.8)	11.6 (10.6, 12.6)	0.001
Number of transferred embryos (%)			0.375
1	125 (12.5)	421 (10.9)	
2	833 (81.3)	3218 (83.1)	
3	67 (6.5)	233 (6.0)	
Blastomere or blastocyst transfer (%)			< 0.001
Blastomere transfer	568 (55.5)	2414 (62.3)	
Blastocyst transfer	453 (44.5)	1458 (37.7)	
The day of development of frozen embryos			0.001
Day 3	747 (56.3)	2887 (61.3)	
Day 4	1 (0.1)	0	
Day 5	468 (35.3)	1530 (23.5)	
Day 6	110 (8.3)	291 (6.2)	
Good-quality rate of the embryo implanted	623 (60.8)	2333 (60.3)	0.759
Teratospermia	91 (8.9)	348 (9.0)	0.913
Oligoasthenozoospermia	135 (13.2)	486 (12.6)	0.596
Oviduct factor	897 (87.5)	3426 (88.5)	0.391
Obstetric outcomes (%)			
Diabetes arising in pregnancy	84 (8.2)	314 (8.1)	0.929
Hypertensive disorder	74 (7.2)	162 (4.2)	< 0.001
Bleeding disorder[a]	12 (1.2)	26 (0.7)	0.105
Gestational cholestasis	3 (0.3)	9 (0.2)	0.729
Polyhydramnios	11 (1.1)	27 (0.7)	0.223
Oligohydramnios	28 (2.7)	93 (2.4)	0.545
Infection	13 (1.3)	47 (1.2)	0.888
Vanish twin	99 (9.7)	408 (10.8)	0.412
Caesarean section[b]	837 (85.9)	3029 (78.4)	< 0.001

Values expressed as *N* (%) or median (1st quartile, 3rd quartile). *P* values are from Wilcoxon rank sum tests for continuous variables and the χ^2 test or Fisher's exact tests for categorical variables

a. Including bleeding due to placenta previa, abruptio placentae, haemorrhage after 20th week of gestation

b. Two cases of twin pregnancy delivery, a fetal vaginal delivery, a fetal caesarean delivery

39.9 weeks) vs. 39.3 weeks (38.4–40.0 weeks); $P = 0.227$) and z-score (0.6 (− 0.1, 1.4) vs. (0.6 (0.0, 1.4), $P = 0.584$) were not different between the two groups. The proportions of infants in each birth weight category and gestational week category were not different between the two groups. The rates of neonatal death, SGA and LGA did not differ between the two groups, as shown in Table 3.

Multiples outcomes

Birth weight (2550 g (2150–2900 g) in AC-FET vs. 2600 g (2350–2900 g) in NC-LPS; $P = 0.023$), gestational age (36.6 weeks (35.3–37.6 weeks) in AC-FET vs. 37.1 weeks (36.1–37.9 weeks) in NC-LPS; $P < 0.001$) and z-score (− 0.5 (− 1.1, − 0.0) in AC-FET vs. − 0.4 (− 1.0, 0.2) in NC-LPS, $P = 0.009$) were higher in the NC-LPS group than in the AC-FET group, as shown in Table 3.

Multivariate logistic regression analysis with adjustment for potential confounders

Multiple logistic regression analysis showed that AC-FET (vs. NC-LPS) was not a significant risk factor for CRP, multiple birth rate or miscarriage rate (Table 4). Regarding the risk of

Table 3 Neonatal outcomes in singletons and multiples

	Singletons			Multiples		
	AC-FET	NC-LPS	*P* value	AC-FET	NC-LPS	*P* value
No. of neonatal	787	2802		476	2140	
Stillbirth fetus	5 (0.6)	4 (0.1)	0.029	8 (1.6)	0	
Pregnancies with a least one live born cycles	782	2798		234[a]	1070	
Gender: Male	411 (52.2)	1502 (53.6)	0.493	272 (58.1)	1142 (53.4)	0.061
Birth weight, g	3400 (3100, 3700)	3400 (3050, 3700)	0.299	2550 (2150, 2900)	2600 (2350, 2900)	0.023
Birth weight category (cases)			0.502			0.245
≤ 1500 g	10 (2.3)	45 (1.6)		29 (6.1)	104 (4.9)	
1500–2500 g	35 (4.5)	112 (4.0)		192 (40.7)	816 (38.2)	
2500 –4000 g	679 (86.8)	2445 (87.4)		251 (53.2)	1216 (56.9)	
> 4000 g	50 (6.4)	196 (7.0)		–	–	
Gestational age, weeks	39.3 (38.4, 40.0)	39.1 (38.4, 39.9)	0.227	36.6 (35.3, 37.6)	37.1 (36.1, 37.9)	< 0.001
Gestational age category (cases)			0.618			< 0.001
< 32 weeks	7 (0.9)	14 (0.5)		40 (8.5)	76 (3.6)	
32–37 weeks	54 (6.9)	188 (6.7)		231 (49.1)	957 (44.9)	
37–42 weeks	716 (92.0)	2583 (92.6)		199 (42.3)	1097 (51.5)	
> 42 weeks	1 (0.1)	5 (0.2)		–	–	
z-score	0.6 (0.0, 1.4)	0.6 (− 0.1, 1.4)	0.584	− .5 (− 1.1, − 0.0)	− 0.4 (− 1.0, 0.2)	0.009
Small-for-gestational age	30 (3.9)	85 (3.1)	0.266	79 (16.9)	376 (18.0)	0.220
Large-for-gestational age	222 (28.9)	747 (27.1)	0.378	13 (2.8)	34 (1.6)	0.085
Early neonatal death	3 (0.4)	2 (0.1)	0.073	5 (1.1)	11 (0.5)	0.188
Late neonatal death	1 (0.1)	0 (0.0)	0.218	4 (0.8)	6 (0.3)	0.089
Infant death	0 (0.0)	1 (0.0)	1.00	2 (0.4)	1 (0.0)	0.086

Values expressed as *N* (%) or median (1st quartile, 3rd quartile). *P* values are from Wilcoxon rank sum tests for continuous variables and the χ^2 test or Fisher's exact tests for categorical variables

a. 8 stillbirth fetus in 4 cycles

ectopic pregnancy (adjusted odds ratio (AOR), 1.738; 95% confidence interval (CI), 1.086–2.781) and LBR (AOR, 0.709; 95% CI, 0.626–0.802), AC-FET was a significant risk factor after adjustment (Table 4). For obstetric outcomes, AC-FET was found to be associated with an increased risk for hypertension disorder (AOR, 1.780; 95% CI, 1.262–2.510) and caesarean section (AOR, 1.507; 95% CI, 1.195–1.900), as shown in Table 4.

The incidences of obstetric complications in singletons and multiples are shown in Supplemental Table 1. Hypertensive disorders were more common among singletons and multiples in the AC-FET group than in the NC-LPS group (5.2 vs. 3.4%, $P = 0.016$ for singletons; 13.9 vs. 6.4%, $P < 0.001$ for multiples). In singletons, bleeding disorders and caesarean delivery were more common in the AC-FET group than in the NC-LPS group (bleeding disorder: 1.3 vs. 0.6%, $P = 0.041$; caesarean delivery: 83.6 vs. 72.3%, $P < 0.001$), and in multiples, polyhydramnios was more prevalent after AC-FET (2.1 vs. 0.4%, $P = 0.013$).

Discussion

In our single centre study, pregnancy, obstetric and neonatal outcomes were compared between the NC-LPS and AC-FET groups to investigate differences in the endometrial preparation schemes. We aimed to clarify that the adverse obstetric outcomes reported previously for frozen embryos, including the increased incidence of hypertension [11–13], macrosomia [2, 16, 17], post-term delivery [15] and caesarean section [15], are caused by the embryo vitrification process or by endometrial injury due to hormonal disorders in patients stemming from endometrial preparation schemes. We noted a higher risk of ectopic pregnancies, hypertension disorder and caesarean section in patients who conceived through AC-FET than in patients who conceived through NC-LPS. Our results provide new insights into the increase in adverse obstetric and perinatal outcomes in pregnancies after FET.

After controlling for these variables in further analyses, we observed no difference in CRP or miscarriage rate between the

Table 4 Logistic regression and linear regression analysis results presenting adjusted odds ratios for the main potential confounders representing outcomes achieved in AC-FET with respect to the NC-LPS group

	Unadjusted (95% CI)	Adjusted (95% CI)	Adjusted *P* value
Logistic regression for clinical outcomes			
Clinical pregnancy rate[a]	0.815 (0.746, 0.890)	0.976 (0.874, 1.089)	0.661
Miscarriage rate[a]	1.248 (1.066, 1.462)	1.172 (0.968, 1.418)	0.104
Ectopic pregnancy rate[a]	2.120 (1.454, 3.092)	1.738 (1.086, 2.781)	0.021
Live birth rate[a]	0.718 (0.619, 0.832)	0.709 (0.626–0.802)	< 0.001
Multiple birth rate[a]	0.792 (0.674, 0.930)	1.655 (0.915, 2.995)	0.096
Logistic regression for obstetric outcomes			
Hypertensive disorder[b]	1.782 (1.341, 2.367)	1.858 (1.383, 2.497)	< 0.001
Caesarean section[c]	1.681 (1.386, 2.038)	1.668 (1.365, 2.038)	< 0.001

Regression coefficients (95% CI) associated with the use of NC-LPS group (NC-LPS group = 0 and AC-FET group = 1)

a. Adjusted for BMI, endometrial thickness (mm) on translate day, basal FSH level (miu/ml), blastomere or blastocyst transfer between AC-FET and NC-LPS groups

b. Adjusted for BMI, endometrial thickness (mm) on translate day, basal FSH level (miu/ml), blastomere or blastocyst transfer, singletons or multiples, the day of development of frozen embryos of the two groups

c. Adjusted for BMI, endometrial thickness (mm) on translate day, basal FSH level (miu/ml), blastomere or blastocyst transfer, the day of development of frozen embryos, singletons or multiples and the incidence of hypertension disorder of the two groups

two groups. However, the ectopic pregnancy rate was higher in the AC-FET group than in the NC-LPS group. The CRP and miscarriage rate were not different between the two groups for patients with regular ovulatory cycles, which confirmed previous findings [20, 22, 33]. The possible reasons for ectopic pregnancies are complicated, including tubal disease, ethnic difference and obstetric history [34–36]. Ectopic pregnancies were also reported to be associated with supraphysiological doses of hormones which impaired endometrial receptivity [36–38], so there is a logical explanation for the increased incidence of ectopic pregnancies during AC-FET. The LBR was lower after AC-FET, which may be due to the slightly higher miscarriage rate and higher ectopic pregnancies rate in this group, although this finding must be confirmed in a randomised, controlled pilot trial.

Interestingly, we found a higher risk of hypertension disorder in mothers of both singletons and multiples after AC-FET than NC-LPS. S. Opdahl [11] and Osamu Ishihara [12] reported that a higher incidence of hypertensive disorders was associated with FET, with a reported incidence of 2.7–2.9% after FET, 5.9% among ART singletons and 12.6% among ART twin pregnancies; these rates are higher than those reported herein for the NC-LPS group and lower than those found in the AC-FET group. Our results indicate a possible link between endometrial preparation and placenta-related diseases. The adverse obstetric outcomes of frozen embryos might be caused by supraphysiological hormone levels during early trophoblast invasion, which lead to abnormal placenta formation [18, 39, 40]. Sex steroids are present in extremely high concentrations in the maternal circulation and are important paracrine and autocrine regulators of a wide range of maternal and placnetal fuctions so that sex steroids modulate uterine-placental vasculature which may influence obstetric and neonatal outcomes [41]. Further experiments are needed to confirm this hypothesis.

Another finding of our study is that the rate of caesarean section was higher in the AC-FET group, which was consistent with the findings reported by Kazuki Saito [15]. In China, the rate of caesarean section has increased dramatically from 18% to almost 50% over the past two decades [42], especially among women who undergo ART. The caesarean section rate in our study was 81.3%, which was consistent with Yang's findings (85.3%) [43]. Because higher risk of hypertension disorder was in AC compared to NC, the endometrial preparation using AC itself or pregnancy complications may be responsible for the higher rate of caesarean section. We found that AC remained a significant risk factor for caesarean section even after adjustment for confounding factors including the incidence of hypertension which shows that the higher incidence of caesarean section may be caused by the AC-FET itself.

The other discovery in our study was that neonatal outcomes in singletons (birthweight, gestational week, z-score) were not significantly different between the AC-FET and NC-LPS groups, which was consistent with the findings of Kazuki Saito [15]. However, in multiples, birth weight, gestational week and z-score were higher in the NC-LPS group. The difference between singletons and multiples may be caused by the greater frequency of pregnancy disorders and adverse outcomes in multiples, including hypertensive disorders, polyhydramnios associated with preterm delivery, lower birth weight and higher perinatal mortality and morbidity [44–46].

Since AC requires medication, the condition might be less physiological than for those with NC, we suggest that if the patients was treated by AC-FET, single embryo transfer was better.

Several limitations associated with the present study warrant mention. First, this was a retrospective observational study. The lack of research on fresh embryo cycles, may contribute to selection bias. In addition, the reason for the use of AC-FET was not analyzable. Second, because of the inherent limitation of the data, we were unable to adjust for potential confounding variables, such as parity, smoking status and alcohol intake, and socioeconomic status. Temporal and geographic bias was also not adjusted, while many clinical factors and procedures potentially changed over time and place. Third, the possibilities of bias and residual confounding factors are always a concern, even after multivariate analysis. Further randomised control trials are needed to verify our findings. However, our results clearly demonstrated the associations between adverse pregnancy outcomes and endometrial preparation by AC, helping to identify those patients who potentially require additional care.

Finally, our results clearly demonstrated the associations between adverse obstetric outcomes and endometrial preparation by AC-FET. Notably, the present study is the first large retrospective analysis comparing NC-LPS with AC-FET and the findings can offer a contribution to guiding clinical practice in FET cycles and further randomised controlled studies are needed to show an effect on outcomes of obstetric and neonatal in FET.

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Authors' contributions GL, GL and FG conceptualised the project. SJ and XL oversaw data collection, analysed the data and drafted the manuscript. SZ provided substantive edits to the manuscript. S.J. and X.L. should be considered co-first authors.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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