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Effect of unplanned spontaneous follicular growth and ovulation on pregnancy outcomes in planned artificial frozen embryo transfer cycles: a propensity score matching study

Yan Su[1,†], Hui Ji[1,2,†], Wei Jiang[1], Lu Xu[1], Jing Lu[1], Chun Zhao[1], Mianqiu Zhang[1], Shanren Cao[1], Xiufeng Ling[1,*], and Rong Shen[1,2,*]

[1]Department of Reproductive Medicine, Women's Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, Nanjing, China, [2]State Key Laboratory of Reproductive Medicine, Nanjing Medical University, Nanjing, China

*Correspondence address. Department of Reproductive Medicine, Women's Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, 123 Tianfeixiang, Mochou Road, Nanjing, Jiangsu 210004, China. E-mail: lingxiufeng_njfy@163.com; State Key Laboratory of Reproductive Medicine, Nanjing Medical University, 101 Longmian Rd, Nanjing, Jiangsu 211166, China. E-mail: rongshen163@163.com

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STUDY QUESTION: Does unplanned spontaneous follicular growth and ovulation affect clinical outcomes after planned artificial frozen-thawed embryo transfer (AC-FET) cycles?

SUMMARY ANSWER: AC-FET and spontaneous follicular growth and ovulation events resulted in notably better pregnancy outcomes with a significantly higher implantation rate (IR), clinical pregnancy rate (CPR), ongoing pregnancy rate (OPR) and live birth rate (LBR) and a significantly lower miscarriage rate.

WHAT IS KNOWN ALREADY: The AC-FET protocol without GnRH agonist administration is associated with a low incidence of follicular growth and ovulation. In the literature, authors often refer to these types of cycles with concern due to possibly impaired FET outcomes. However, the real impact of such cycles has yet to be elucidated due to the lack of existing data.

STUDY DESIGN, SIZE, DURATION: This was a retrospective clinical study involving 2256 AC-FET cycles conducted between January 2017 and August 2019. Propensity score (PS) matching was used to control for confounding variables.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Subjects were divided into two groups: a study group: cycles with spontaneous follicular growth and ovulation (the maximum diameter of follicles in any ovary was $\geq$14 mm and ovulation was confirmed by consecutive ultrasound examinations) and a control group featuring cycles without growing follicles (the maximum diameter of follicles in both ovaries were $<$10 mm). The study group was matched by PS with the control group at a ratio of 1:2. The study group consisted of 195 patients before PS matching and 176 patients after matching. The numbers of participants in the control group before and after PS matching were 2061 and 329, respectively.

MAIN RESULTS AND THE ROLE OF CHANCE: This analysis showed that patient age (adjusted odds ratio [aOR] 1.05; 95% CI 1.01–1.09; P=0.010) and basal FSH level (aOR 1.06; 95% CI 1.01–1.11; P=0.012) were significantly and positively related with the spontaneous follicular growth and ovulation event. In addition, this event was negatively correlated with BMI (aOR 0.92; 95% CI 0.87–0.97; P=0.002), AMH level (aOR 0.66; 95% CI 0.59–0.74; P<0.001) and a high starting oestrogen dose (aOR 0.53; 95% CI 0.38–0.76 for 6 mg vs. 4 mg; P<0.001). Baseline characteristics were similar between groups after PS matching. Patients in the study group had a significantly higher IR (28.8% vs. 21.8%, P=0.016), CPR (44.9% vs. 33.4%, P=0.011), OPR (39.2% vs. 26.1%, P=0.002) and LBR (39.2% vs. 24.9%, P=0.001) and a lower miscarriage rate (12.7% vs. 25.5%, P=0.030), compared with those in the control group.

[†]Su and Ji are joint first authors.

LIMITATIONS, REASONS FOR CAUTION: This was a retrospective study carried out in a single centre and was therefore susceptible to bias. In addition, we only analysed patients with normal ovulation patterns and excluded those with follicular growth but without ovulation. Further studies remain necessary to confirm our results.

WIDER IMPLICATIONS OF THE FINDINGS: It is not necessary to cancel cycles that experience spontaneous follicular growth and ovulation. Our data support promising clinical outcomes after this event. Our findings are important as they can better inform clinicians and patients.

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Key words: frozen-thawed embryo transfer / planned artificial cycle / unplanned spontaneous follicular growth and ovulation / pregnancy outcomes / endogenous progesterone / propensity score matching

Introduction

Due to improvements in cryopreservation techniques and the trend towards transferring fewer embryos during fresh *in-vitro* fertilization (IVF) cycles, the number of frozen-thawed embryo transfer (FET) cycles has steadily increased (European IVF-monitoring Consortium for the European Society of Human Reproduction and Embryology *et al.*, 2016; Bai *et al.*, 2020). In addition, FET has been extensively used either to reduce the risk of ovarian hyperstimulation syndrome for hyper-responders or to perform preimplantation genetic testing (Roque *et al.*, 2019). Data demonstrate that FET is a feasible option for maintaining both safety and a favourable success rate (Zhang *et al.*, 2018; Roque *et al.*, 2019).

The first key step in FET treatment is preparing an endometrium with good receptivity by the application of different cycle regimens. To date, there is no consensus on the optimal protocol to prepare the endometrium for a preimplantation embryo (Ghobara *et al.*, 2017; Mackens *et al.*, 2017). The artificial cycle (AC), one of the commonly used protocols among all regimens, is safe, effective and flexible, with a high level of control and a low cycle cancellation rate. Using this protocol, the endometrium is prepared with exogenous oestrogen and progesterone to mimic the natural cycle (NC). To prevent follicular recruitment and to avoid spontaneous follicular growth or ovulation, oestrogen is usually administered at a higher dosage (4 or 6 mg of oestradiol valerate per day) and is taken early in the menstrual cycle (Day 1, 2 or 3). However, unplanned spontaneous follicular growth and ovulation can still occur, the incidence of which ranges from 1.9% (van de Vijver *et al.*, 2014) to 7.4% (Dal Prato *et al.*, 2002) without pituitary suppression using GnRH agonist. According to the literature, artificial frozen-thawed embryo transfer (AC-FET) cycles with spontaneous follicular growth and ovulation should be cancelled (Ubaldi *et al.*, 1997; El-Toukhy *et al.*, 2004; Dal Prato and Borini, 2006). As a result, numerous cycles might be lost, thus affecting patients financially and emotionally.

A pertinent question, therefore, is whether unplanned follicular growth and ovulation really does have a negative impact on the overall clinical outcomes in AC-FET cycles. There is currently no data regarding this issue. Therefore, we decided to focus particularly on the unplanned spontaneous follicular growth and ovulation phenomenon in planned AC-FET cycles and conducted this retrospective study to investigate the possible variables that might lead to this event and the association between this event and pregnancy outcomes.

Materials and methods

Study design

The Ethics Committee of Nanjing Maternity and Child Health Care Hospital approved this study (NJFY-2020-KY-052). The study was also carried out in accordance with the Declaration of Helsinki. We conducted this retrospective study from January 2017 to August 2019 at the reproductive centre of the hospital. Since the study was retrospective and analysed patient data anonymously, informed patient consent was not required.

We included all patients undergoing FET cycles with an AC regimen without GnRH agonist down regulation. All participants had at least one cleavage stage embryo or blastocyst cryopreserved following IVF or intracytoplasmic sperm injection insemination. The exclusion criteria for both groups were as follows: (i) the presence of uterus malformation or fallopian hydrosalpinx; (ii) cycles involving oocyte donation or oocyte vitrification; (c) embryos undergoing preimplantation genetic testing; and (d) cases involving missing cycle data or follow-up.

FET protocols, embryo transfer and luteal phase support

After excluding early follicular recruitment or the presence of an ovarian cyst, patients received 4 or 6 mg (2 or 3 mg twice) of oral oestrogen (oestradiol valerate, progynova, Bayer, France) per day on the second or third day of the menstrual cycle for 1 week. Decisions regarding the oestrogen-starting dose were based on physician preference. We then monitored endometrial thickness (EMT) using serial transvaginal ultrasonography and serum assays of oestradiol (*E*2) and progesterone (*P*) levels. If there were no growing follicles (the maximum diameter of follicles in both ovaries was $<$10 mm), we adjusted the oestrogen dose to 6–10 mg per day, as determined by EMT and serum *E*2 levels. When the EMT reached 7 mm, serum *E*2 level peaked at 200 pg/ml, and the serum levels of P were $<$1.5 ng/ml, we initiated luteal phase support (LPS) (P+1). In cases where a growing follicle emerged (the maximum diameter of the follicle was $>$10 mm), patients returned for follow-up 2–3 days later. If the dominant follicles disappeared or stopped growing, the cycles remained as AC-FET and the choice of LPS starting day was based on EMT and hormone levels. If the growing follicles further developed to larger than 14 mm in mean diameter, the AC-FET was changed to 'natural cycles'. Patients continued to take the same oestrogen dosage to minimize the incidence of withdrawal vaginal bleeding. We also advised these patients to suspend sexual intercourse during the whole treatment. Once the dominant follicle reached 14 mm, serum LH, *E2* and *P* levels were measured on the same day. Based on our previous data and experience, the LH surge was defined as a LH level $\geq$20 mIU/ml. When LH was $\geq$20 mIU/ml, we carried out ultrasound every day and measured serum hormone levels (LH, *E2* and *P*) every 1–2 days. In some circumstances, the dominant follicle ovulated spontaneously before reaching 18 mm. We recruited patients if the peak *E2* level was $\geq$200 pg/ml, *P* was <1.5 ng/ml before ovulation, and the EMT was $\geq$7 mm. If the leading follicle was $\geq$18 mm and EMT $\geq$7 mm with *E2* $\geq$200 pg/ml and *P*<1.5 ng/ml, regardless of the LH level, we injected 10 000 IU of hCG (Lizhu, China). Ultrasound was applied to detect ovulation everyday thereafter. LPS was started after ultrasound confirmed that the leading follicle had been ovulated (*P*+1). Cycles were cancelled if serum *P* level was >1.5 ng/ml prior to LPS, or if a prolonged period of oestrogen priming (more than 21 days) was required.

Therefore, the patients were divided into two groups according to the development of follicles. The study group featured cycles involving spontaneous follicular growth and ovulation (the maximum diameter of follicles in any ovary was $\geq$14 mm and ultrasound examination confirmed that ovulation had successfully occurred). The control group involved cycles without a growing follicle (the maximum diameter of follicles in both ovaries was <10 mm). Both groups (the study group with follicle growth and the control group without endogenous progesterone) received the same LPS: 90 mg of vaginal progesterone (Crinone, Merck Serono, UK) once per day and 10 mg of dydrogesterone (Abbott Biologicals B.V., the Netherlands) three times daily. These treatments were continued until 14 days after FET. If the pregnancy test was positive, then LPS was continued until 10–12 weeks of pregnancy.

Embryos were vitrified and warmed based on protocols that were described previously (Chen *et al.*, 2014). Specifically, after surviving the warming procedure, post-thaw day 3 (D3) embryos were cultured at 37°C in a 6% CO_2, 5% O_2 and 89% N_2 incubator for another 16 h before transfer; this practice was required due to our work schedule. For Day 5 (D5) or Day 6 (D6) blastocysts, an additional 2–6 h incubation was performed before transfer. D3 embryos reaching the morula stage were defined as good-quality embryos (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011) and transferred 3 days after progesterone administration (*P*+4). D5 or D6 blastocysts at Stage 3 or above, with at least one score B for either inner cell mass (ICM) or trophectoderm (TE), were selected for vitrification (Gardner and Schoolcraft, 1999). Prior to vitrification, good-quality blastocysts were scored as larger than 3BB (grade 3–6 AA/AB/BA/BB) according to the cavity expansion level, along with ICM and TE. All D5 or D6 blastocyst transfers were performed on the sixth day of progesterone exposure (*P*+6).

Outcome measures

Levels of β-hCG were tested 2 weeks after FET. Clinical pregnancy was confirmed by ultrasonic identification of the gestational sac after 6 weeks of pregnancy. Early miscarriage was defined as foetal loss prior to gestational week 12. Miscarriage was termed as the loss of a spontaneous clinical pregnancy prior to 28 weeks of gestation. Ongoing pregnancy was defined as a successful clinical pregnancy that had progressed beyond 12 weeks of gestation. Live birth was considered when a living foetus was born after 28 weeks of pregnancy. Implantation rates (IRs) were calculated as the ratio of the gestational sac number over the number of embryos transferred. The primary outcome was live birth rate (LBR). The secondary outcomes included IR, clinical pregnancy rate (CPR), ongoing pregnancy rate (OPR), miscarriage rate and early miscarriage rate.

Propensity score matching and statistical analysis

All data were analysed with SPSS 24.0 (IBM, NY, USA) embedded with a 1: n propensity score (PS) matching plug-in. Comparisons of descriptive statistics between groups were analysed by the independent samples *t*-test and the Mann–Whitney U test (if data were not normally distributed). Following the *t*-test, continuous data are presented as the mean±SD, while data derived from the U test are presented as the median (first quartile, third quartile). Pearson's chi-square test or Fisher's exact test were used for categorical data. Multivariate logistic regression analysis was conducted to identify the factors that had a significant influence on spontaneous follicular growth and ovulation event in AC cycles. The variables that showed significant differences on univariate analysis at *P*<0.05 or had significant effect on LBR were included in the multiple regression model. *P*<0.05 was considered to be statistically significant.

To compare FET outcomes between the study group and the control group, we used PS matching to adjust data for potential confounding factors and selection bias. The PS was determined using multivariate logistic regression. Final variables included age, infertility type, duration and cause of infertility, BMI, basal FSH, anti-Müllerian hormone (AMH), embryo transfer rank, EMT, embryo developmental stage (D3 or D5/D6), number of transferred embryos and good-quality embryo transferred (yes or no). After calculating the PS of each subject, patients in the study group were matched in a 1:2 ratio to those in the control group using nearest neighbour matching with a 0.1 calliper width. Furthermore, based on the final results of a 14% difference in LBR between the two groups, post hoc power analysis (two-sided α of 0.05, power of 0.80) indicated that 171 cycles were needed in each group to detect an increment in LBR (primary outcome) from 25% to 39%.

Results

Before PS matching

Figure 1 shows a flow chart of the study population. From a total of 2505 AC-FET cycles, 2256 met the inclusion criteria. Of these, 195 were included in the study group and 2061 were in the control group. Baseline characteristics are presented in Table I. Compared with the control group, patients in the study group were significantly older (35.3±6.1 vs. 30.6±4.8 years, *P*<0.001), had a significantly lower BMI (22.1±3.0 vs. 22.6±3.2 kg/m^2, *P*=0.034) and AMH level (1.1 (0.5–2.4) vs. 4.1 (2.4–6.8) ng/ml, *P*<0.001) and a significantly higher basal FSH level (10.0±4.1 vs. 7.6±2.7 mIU/ml, *P*<0.001). There were also significant differences in terms of the type, duration and cause of infertility

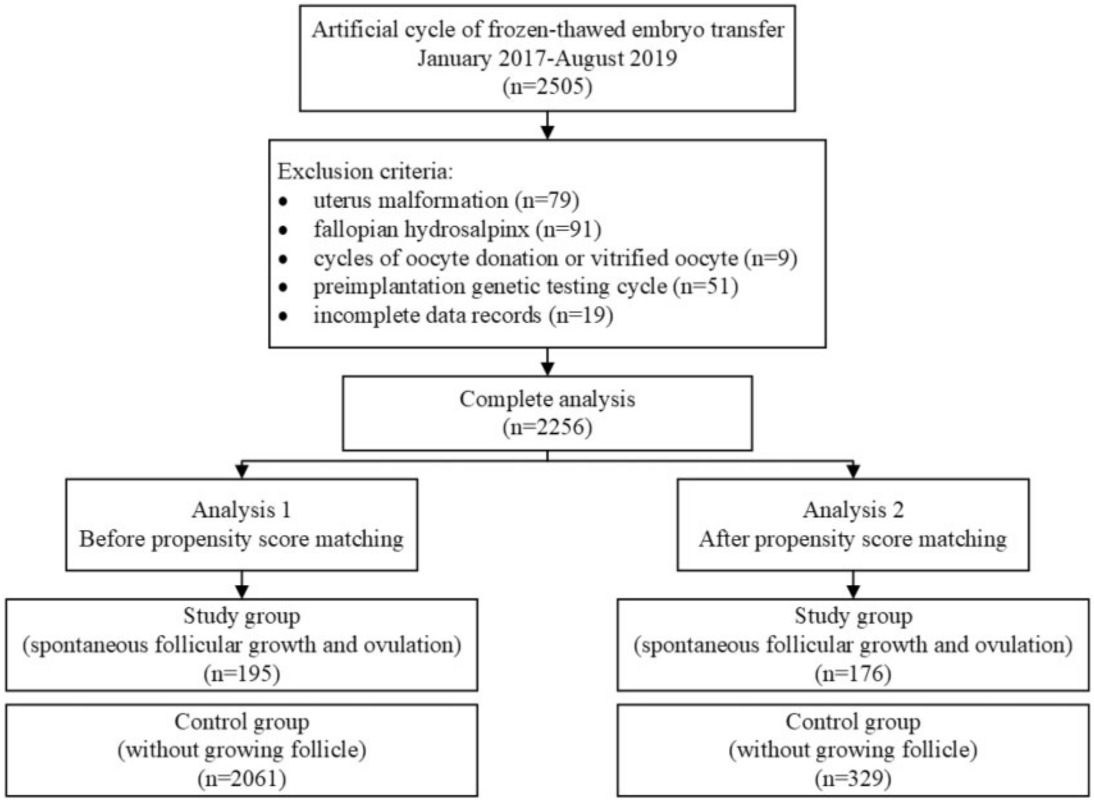

Figure 1. **Flow chart.**

(P<0.05) when compared between the two groups. Women in the study group were significantly more likely to start on 4 mg of oral oestrogen than those in the control group (35.4% vs. 24.2%, P=0.001). Additional subgroup analysis was carried out for female age; in the study group, we observed a significantly lower portion of patients aged <35 years (48.2% vs. 82.1%, P<0.001) than in the control group.

Multivariable logistic regression analysis was used to predict potential confounders for the occurrence of the follicular growth and ovulation event (Table II). The included parameters were as follows: age, infertility type (primary vs. secondary), duration of infertility (1–2 vs. 3–5 vs. ≥6), cause of infertility (tubal factor vs. ovulatory dysfunction vs. diminished ovarian reserve (DOR) or advanced maternal age (AMA) vs. endometriosis vs. male factor vs. unknown factor), BMI, basal FSH, AMH and oestrogen-starting dose (4 vs. 6 mg). Variables that showed a significant influence on this event were age (adjusted odds ratio [aOR]=1.05; 95% CI: 1.01–1.09, P=0.010), BMI (aOR=0.92; 95% CI: 0.87–0.97; P=0.002), basal FSH (aOR=1.06; 95% CI: 1.01–1.11; P=0.012), AMH (aOR=0.66; 95% CI: 0.59–0.74; P<0.001) and oestrogen-starting dose (aOR=0.53; 95% CI: 0.38–0.76 for 6 vs. 4 mg subgroup; P<0.001).

After PS matching

After PS matching, the study group consisted of 176 patients who matched 329 patients in the control group. We compared the baseline characteristics, transferred embryo information, and clinical outcomes between the two groups. Basic parameters were not statistically different (Table III). Table IV demonstrates that the study group had a significantly higher IR (28.8% vs. 21.8%, P=0.016), CPR (44.9% vs. 33.4%, P=0.011), OPR (39.2% vs. 26.1%, P=0.002) and LBR (39.2% vs. 24.9%, P=0.001). It also depicted a lower early miscarriage rate in the study group, although the difference between groups was not significant (12.7% vs. 21.8%, P=0.106). Nevertheless, the miscarriage rate before gestational week 28 was significant lower in the study group when compared with that in the control group (12.7% vs. 25.5%, P=0.030).

Subgroup analysis

Of the 195 cycles showing unplanned follicular growth and ovulation, women were allocated to one of two groups based on whether hCG was injected or not: a non-triggering group (n=66) and a triggering group (n=129). The demographic and clinical characteristics of the two groups are summarized in Table V. The maximum follicle size before ovulation in the non-triggering group was, as expected, significantly smaller than that in the triggering group (16.1±1.1 vs. 19.3±1.1 mm, P < 0.001). Other basic variables were comparable between the two groups. There were no differences either in regard to IR, CPR, OPR, miscarriage rate and early miscarriage rate (P > 0.05). In addition, no statistical difference was observed between the two groups in terms of LBR (24/66 (36.4%) vs. 51/129 (39.5%), P = 0.667).

Table I Patient demographic characteristics before propensity score matching.

Characteristic	Study group (n = 195)	Control group (n = 2061)	P-value
Age (years)[a]	35.3±6.1	30.6±4.8	<0.001
Age (<35 years), n (%)[c]	94 (48.2)	1692 (82.1)	<0.001
Infertility type, n (%)[c]			<0.001
Primary	65 (33.3)	1131 (54.9)	
Secondary	130 (66.7)	930 (45.1)	
Duration of infertility (years), n (%)[c]			0.016
1–2	84 (43.1)	967 (46.9)	
3–5	64 (32.8)	763 (37.0)	
≥6	47 (24.1)	331 (16.1)	
Cause of infertility, n (%)[c]			<0.001
Tubal factor	83 (42.6)	1257 (61.0)	
Ovulatory dysfunction	1 (0.5)	103 (5.0)	
DOR or AMA	73 (37.4)	176 (8.5)	
Endometriosis	9 (4.6)	38 (1.8)	
Male factor	22 (11.3)	402 (19.5)	
Unknown factor	7 (3.6)	85 (4.1)	
BMI (kg/m^2)[a]	22.1±3.0	22.6±3.2	0.034
Basal FSH (mIU/ml)[a]	10.0±4.1	7.6±2.7	<0.001
AMH (ng/ml)[b]	1.1 (0.5–2.4)	4.1 (2.4–6.8)	<0.001
Oestrogen-starting dose (mg), n (%)[c]			0.001
4	69 (35.4)	499 (24.2)	
6	126 (64.6)	1562 (75.8)	

DOR, diminished ovarian reserve; AMA, advanced maternal age; AMH, anti-Müllerian hormone.
[a]Data are expressed as mean±SD for continuous variables following normal distribution.
[b]Data are expressed as median (first quartile, third quartile) for continuous variables not normally distributed.
[c]Data are expressed as numbers (percentage) for categorical variables.

Table II Variables associated with spontaneous follicular growth and ovulation event in artificial frozen-thawed embryo transfers: multivariate logistic regression analysis.

Variable	aOR	95% CI	P-value
Age (years)	1.05	1.01–1.09	0.010
Infertility type			0.212
Primary	Reference		
Secondary	1.27	0.87–1.86	
Duration of infertility (years)			0.267
1–2	Reference		
3–5	1.20	0.82–1.74	0.345
≥6	1.42	0.92–2.19	0.113
Cause of infertility			0.517
Tubal factor	Reference		
Ovulatory dysfunction	0.39	0.05–2.91	0.360
DOR or AMA	1.15	0.70–1.87	0.584
Endometriosis	1.89	0.82–4.34	0.133
Male factor	0.85	0.51–1.41	0.524
Unknown factor	1.21	0.52–2.82	0.654
BMI (kg/m^2)	0.92	0.87–0.97	0.002
Basal FSH (mIU/ml)	1.06	1.01–1.11	0.012
AMH (ng/ml)	0.66	0.59–0.74	<0.001
Oestrogen-starting dose (mg)			<0.001
4	Reference		
6	0.53	0.38–0.76	

[a]OR, adjusted odds ratio.

Multivariate analysis for LBR was performed to adjust for the following potential confounders: female age, duration of infertility, BMI, basal FSH, AMH, maximum follicle size, EMT, embryo developmental stage (D3 vs. D5/D6), number of transferred embryos, good-quality embryo transferred (yes vs. no) and hCG injection (yes vs. no). The hCG injection had no significant negative impact on LBR (aOR=0.66; 95% CI: 0.20–2.18; P=0.494).

Discussion

It is well known that the AC protocol provides more flexibility and freedom when planning FET. Exogenous oestrogen is used in AC-FET cycles to mimic the follicular phase of NC while progesterone is used to imitate the secretory phase. The administration of oestrogen is also necessary to suppress follicular recruitment and growth. Although unplanned spontaneous follicular growth and ovulation is a rare condition in AC-FET, it can occur (Dal Prato et al., 2002; van de Vijver et al., 2014; Groenewoud et al., 2016). Due to fear associated with impaired FET outcomes, the literature usually describes unplanned follicular growth and ovulation as an adverse circumstance. Cycles are therefore normally cancelled in these circumstances because it is difficult to determine the appropriate time for transfer as serum progesterone might increase early (Dal Prato and Borini, 2006), the rising LH levels could adversely interfere with the receptive window (El-Toukhy et al., 2004) and ovulation during hormonal supplementation may lead to embryo-endometrial asynchrony (Ubaldi et al., 1997). In order to diminish the chance of this unpredictable situation, exogenous oestrogen is usually administered at a high dose (i.e. 6 mg) starting from the early menstrual cycle onwards (i.e. Days 1–3) (Simon et al., 1998) or is applied after a GnRH agonist (Groenewoud et al., 2013; Berkkanoglu et al., 2017). Concerning the low incidence of follicle growth and ovulation in AC-FET, no clinical studies have attempted to investigate the correlation between this event and FET results so far. As such, there is considerable debate on this matter.

In this study, we collected data relating to this special event with two specific aims: first, to identify the factors involved, and second, to determine whether this particular event really does have negative consequences in AC-FET cycles. Over a period of almost three years (January 2017–August 2019), we were able to recruit 2256 patients (195 in the study group and 2061 in the control group). Patients in the study group were older and had a lower body weight than those in the control group. In addition, the study group had significantly higher

Table III Patient demographic characteristics after propensity score matching.

Characteristic	Study group (n=176)	Control group (n=329)	P-value
Age (years)[a]	34.7±5.9	34.5±6.1	0.770
Age (<35 years), n (%)[c]	93 (52.8)	173 (52.6)	0.681
Infertility type, n (%)[c]			0.580
Primary	63 (35.8)	126 (38.3)	
Secondary	113 (64.2)	203 (61.7)	
Duration of infertility (years), n (%)[c]			0.844
1–2	74 (42.0)	146 (44.4)	
3–5	62 (35.2)	108 (32.8)	
≥6	40 (22.7)	75 (22.8)	
Cause of infertility, n (%)[c]			0.998
Tubal factor	80 (45.5)	146 (44.4)	
Ovulatory dysfunction	1 (0.6)	3 (0.9)	
DOR or AMA	59 (33.5)	110 (33.4)	
Endometriosis	8 (4.5)	15 (4.6)	
Male factor	21 (11.9)	42 (12.8)	
Unknown factor	7 (4.0)	13 (4.0)	
BMI (kg/m^2)[a]	22.2±3.1	22.3±3.0	0.795
Basal FSH (mIU/ml)[a]	9.5±1.9	9.5±1.6	0.813
AMH (ng/ml)[b]	1.2 (0.6–2.5)	1.6 (0.7–3.1)	0.091
Embryo transfer rank, n (%)[c]			0.819
1	107 (60.8)	200 (60.8)	
2	47 (26.7)	98 (29.8)	
3	15 (8.5)	20 (6.1)	
4	6 (3.4)	9 (2.7)	
5	1 (0.6)	2 (0.6)	
Endometrial thickness (mm)[a]	9.4±1.9	9.5±1.6	0.741
Embryo developmental stage, n (%)[c]			0.766
D3	117 (66.5)	223 (67.8)	
D5/D6	59 (33.5)	106 (32.2)	
Number of transferred embryos[a]	1.9±0.5	1.9±0.6	0.847
Good-quality embryo transferring, n (%)[c]	93 (52.8%)	176 (53.5)	0.888

D3, Day 3, cleavage stage embryo; D5/D6, Day 5/Day 6, blastocyst.
A good-quality embryo was defined as a morula stage embryo for D3 embryos or a grade 3–6 embryo ≥BB (AA, AB, BA, BB) for blastocysts.
[a]Data are expressed as mean±SD for continuous variables following normal distribution.
[b]Data are expressed as median (interquartile range) for continuous variables not normally distributed.
[c]Data are expressed as numbers (percentage) for categorical variables.

Table IV Pregnancy outcomes between the study group and the control group after propensity score matching.

Outcomes	Study group (n=176)	Control group (n=329)	P-value
Implantation rate, n/N (%)	95/330 (28.8)	135/620 (21.8)	0.016
Clinical pregnancy rate, n/N (%)	79/176 (44.9)	110/329 (33.4)	0.011
Early miscarriage rate, n/N (%)	10/79 (12.7)	24/110 (21.8)	0.106
Miscarriage rate, n/N (%)	10/79 (12.7)	28/110 (25.5)	0.030
Ongoing pregnancy rate, n/N (%)	69/176 (39.2)	86/329 (26.1)	0.002
Live birth rate, n/N (%)	69/176 (39.2)	82/329 (24.9)	0.001

basal levels of FSH and lower levels of AMH compared with the control group. Furthermore, the proportion of patients taking a 4 mg starting dose of oestrogen was statistically higher in the study group (35.4% vs. 24.2%, P=0.001). Logistic regression analysis showed that older patients with a lower BMI and AMH level, higher basal FSH levels and a starting oestrogen dose of 4 mg per day were most likely to experience unplanned follicular growth and ovulation in AC treatments.

Our knowledge of the selection window indicates that during the luteal-follicular transition, a small increase in FSH levels is responsible for the selection of a single dominant follicle that will ultimately undergo ovulation (Schipper *et al.*, 1998). Women with DOR or AMA may experience shorter menstrual cycles and a premature rise of FSH ~2 days prior to the onset of menstruation; this continues throughout the next early follicular phase (Sefrioui *et al.*, 2019). This may accelerate the development of some sensitive follicles during the late luteal phase and spontaneous follicular growth could occur, even after the application of a high oestrogen dose from the early phase of the menstrual cycle. It is understandable that this event happens more frequently in patients who are older or have higher basal FSH levels and lower AMH levels. We further divided our participants into two subgroups based on BMI value: those with a BMI <25 kg/m^2 and those with a BMI ≥25 kg/m^2. The '< 25' subgroup had significantly higher basal FSH level than the '≥ 25' subgroup (8.0±3.0 vs. 7.2±2.3 mIU/ml, P<0.001); this may be the reason underlying the higher incidence of the follicle development event in patients with a lower BMI. In a previous study, Simon *et al.* (1998) found that a 6-mg daily starting dose of 17β-oestradiol could successfully prevent the occurrence of follicular dominance when compared with a GnRH agonist combined with 4 mg of 17β-oestradiol. In this regard, we also observed a significantly higher incidence of the spontaneous event in patients taking 4 mg of oestrogen per day than those taking 6 mg per day.

No consensus has yet been reached on the effect of follicular development and ovulation on pregnancy results. Our study revealed that women in the study group not only experienced higher rates of IR, CPR, OPR and LBR, but also a significantly lower miscarriage rate when compared with those in the control group. We initially considered that the two groups had comparable clinical outcomes and the fact that the study group had promising pregnancy results was beyond our original expectations. When considered in more depth, particularly with regard to the comparable baseline characteristics and the similar LPS, the biggest difference between the groups appeared to be the spontaneous follicular growth and ovulation event in the study group. In NC, the existing corpus luteum remaining after ovulation can secret an adequate supply of natural progesterone to induce the secretory transformation of the endometrium, lay the foundation for a receptive endometrium and maintain pregnancy (Practice Committee of the

Table V The demographic and clinical characteristics between the non-triggering and triggering group for patients with spontaneous follicle growth and ovulation.

Characteristic	Non-triggering group (n=66)	Triggering group (n=129)	P-value
Age (years)[a]	35.7±6.0	35.1±6.2	0.469
Age (<35 years), n (%)[c]	32 (48.5)	62 (48.1)	0.955
Infertility type, n (%)[c]			0.335
Primary	19 (28.8)	46 (35.7)	
Secondary	47 (71.2)	83 (64.3)	
Duration of infertility (years), n (%)[c]			0.054
1–2	25 (37.9)	59 (45.7)	
3–5	29 (43.9)	35 (27.1)	
≥6	12 (18.2)	35 (27.1)	
Cause of infertility, n (%)[c]			0.348
Tubal factor	23 (34.8)	60 (46.5)	
Ovulatory dysfunction	0 (0.0)	1 (0.8)	
DOR or AMA	27 (40.9)	46 (35.7)	
Endometriosis	2 (3.0)	7 (5.4)	
Male factor	11 (16.7)	11 (8.5)	
Unknown factor	3 (4.5)	4 (3.1)	
BMI (kg/m^2)[a]	21.7±3.2	22.3±2.9	0.187
Basal FSH (mIU/ml)[a]	10.7±4.8	9.7±3.7	0.121
AMH (ng/ml)[b]	1.1 (0.6–2.2)	1.2 (0.4–2.6)	0.836
Embryo transfer rank, n (%)[c]			0.938
1	39 (59.1)	75 (58.1)	
2	18 (27.3)	37 (28.7)	
3	6 (9.1)	12 (9.3)	
4	3 (4.5)	4 (3.1)	
5	0 (0.0)	1 (0.8)	
Maximum follicle size (mm)[a]	16.1±1.1	19.3±1.1	<0.001
Endometrial thickness (mm)[a]	9.0±1.7	9.5±1.9	0.081
Embryo developmental stage, n (%)[c]			0.191
D3	50 (75.8)	86 (66.7)	
D5/D6	16 (24.2)	43 (33.3)	
Number of transferred embryos[a]	2.0±0.5	1.9±0.5	0.262
Good-quality embryo transferring, n (%)[c]	32 (48.5)	57 (44.2)	0.568
Pregnancy outcome			
Implantation rate, n/N (%)	34/129 (26.4)	70/241 (29.0)	0.583
Clinical pregnancy rate, n/N (%)	26/66 (39.4)	60/129 (46.5)	0.344
Early miscarriage rate, n/N (%)	1/26 (3.8)	9/60 (15.0)	0.270
Miscarriage rate, n/N (%)	2/26 (7.7)	9/60 (15.0)	0.492
Ongoing pregnancy rate, n/N (%)	25/66 (37.9)	51/129 (39.5)	0.822
Live birth rate, n/N (%)	24/66 (36.4)	51/129 (39.5)	0.667

[a]Data are expressed as mean±SD for continuous variables following normal distribution.
[b]Data are expressed as median (interquartile range) for continuous variables not normally distributed.
[c]Data are expressed as numbers (percentage) for categorical variables.

American Society for Reproductive Medicine, 2012; Lawrenz et al., 2019). However, there is no corpus luteum formation in ACs and exogenous progesterone supplementation is a necessity for LPS. Moreover, the function of the corpus luteum may decrease with declining ovarian reserve or advancing maternal age (Pfister et al., 2019), the infertile aetiology of which has accounted for one-third of participants after PS matching in our study. With the adequate exposure of progesterone, from both endogenous and exogenous LPS, participants in the study group may experience a much higher CPR and a lower miscarriage rate than those who only received exogenous progesterone. Our data were in line with the recent literatures and added to the existing evidence to suggest a potential importance of the corpus luteum during pregnancy, especially for women with poor ovarian reserve (Conrad and Baker, 2013; von Versen-Hoynck et al., 2019; Wang et al., 2020).

A successful implantation depends on the synchronous interaction between a competent embryo and a receptive endometrium. The window of implantation (WOI) only remains for a short period of time (Bergh and Navot, 1992) and has yet to be precisely described for different endometrial preparation protocols (Mackens et al., 2017). Any clinical interventions might cause WOI changes in aspects of opening, closing, length and functionality (Mackens et al., 2017). The WOI might be detected more easily, and be more fully established after follicular ovulation; this may be another reason for the higher IR and CPR in the study group.

Over recent years, researchers have discovered that NC-FET results in better pregnancy outcomes in women with regular menstruation when compared to AC-FET (Guan et al., 2016; Orvieto et al., 2016; Wang et al., 2020). In addition, a previous retrospective analysis reported a higher miscarriage rate after AC compared with NC (Tomas et al., 2012). In a basic research study, Altmae et al. (2016) compared the endometrial transcriptome of 15 biopsy samples during the embryo implantation window between NC and AC protocols and discovered that oestrogen and progesterone supplementation in AC have a negative effect on the expression of genes and pathways that are crucial for endometrial receptivity. In this study, less exogenous oestrogen was applied when growing follicles were presented in the study group. An excessive oestrogen environment, or a suboptimal ratio between oestrogen and progesterone in the control group, could be one possible explanation for the higher pregnancy loss. Although, the study by Altmae et al. (2016) only enrolled recurrent implantation failure patients and might not be extrapolated to the whole IVF population, their finding still provides some evidence in this field.

With regards to the 195 unplanned follicle growth and ovulation cycles analysed, it was evident that the timing of embryo thawing and transfer in NC-FET was in accordance with the observation of ovulation. A distinction was made between the true natural cycle (tNC) and the modified natural cycle (mNC), similar to our non-triggering and triggering group. The optimal strategy has yet to be elucidated and will be debated in the future. A previous RCT (Fatemi et al., 2010) and a large retrospective study (Montagut et al., 2016) demonstrated that the tNC-FET group had better pregnancy outcomes compared to the mNC-FET group. However, other studies have failed to support the superiority of tNC-FET over mNC-FET cycles (Weissman et al., 2009; Chang et al., 2011; Weissman et al., 2011; Tomas et al., 2012; Groenewoud et al., 2017). Although a discussion of this topic is beyond the scope of our article, our data indeed demonstrated that the two groups had similar pregnancy results with regards to IR, CPR, OPR, miscarriage rate and LBR. The designated time of embryo thawing in our research was not solely based on LH level or follicle size. Instead, we applied strict hormonal and follicular development monitoring. LPS was commenced immediately after ovulation was detected to provide the same level of embryo-endometrial synchrony in the two populations. Additionally, all of the patients received LPS, which, collectively, have led to a comparable clinical outcome. Nevertheless, our results should be interpreted with caution as the sample size (66 vs. 129) might be insufficient to detect any statistical difference.

Another interesting, but also important, issue is that patients who had spontaneous follicle growth in AC cycles might conceive spontaneously if they had unprotected intercourse. Literature describes dizygotic twinning after single embryo transfer (Bavan and Milki, 2019). In our current study, patients were discouraged to partake in intercourse throughout the process to eliminate potential infection of the reproductive tract. Therefore, no natural cases of conception were observed. Obviously, multifoetal gestation is associated with an increase in pregnancy complications and neonatal morbidity (American College of Obstetricians and Gynecologists and Society for Maternal-Fetal Medicine, 2014). It may be worthwhile for physicians to caution patients against unprotected intercourse during FET treatment when a growing follicle has emerged.

To the best of our knowledge, this research is the first clinical study focussing on the spontaneous follicular growth and ovulation event in AC-FET cycles. The strength of our study lies in the fact that we have a sufficient sample size to create 80% statistical power. Furthermore, PS matching was used to control for potential confounding between the two groups, thus making the outcomes independent from the different baseline characteristics. Irrespective of its low incidence and the cancellation of cycles, we identified an effective and valid approach to address follicle growth in cycles involving AC-FET. Our study showed that if spontaneous follicular growth was observed, there is no need to cancel the cycle. Following careful monitoring, accurate WOI detection, and sufficient LPS, the clinical outcomes could be even better for women experiencing unplanned follicular growth and ovulation. On the basis of this results, our daily clinical practice has been changed. Patients with DOR or AMA are now administered with 6 mg oestradiol valerate per day. Even if an emerging follicle after higher oestrogen doses appears, the cycles will not be cancelled and are performed cautiously in accordance with the above-mentioned scheme.

There are some limitations to our study that need to be considered. First, this was a retrospective study conducted on a dataset from a single IVF centre. Second, it is possible that our analysis may have been subject to inclusion, selection and statistical bias. Third, we only analysed patients with a normal pattern of ovulation instead of those with follicular growth but no ovulation, which might have different FET outcomes. Further research now needs to be carried out to verify our results; such studies should involve a large sample size.

Conclusion

We found that cases of spontaneous follicular growth and ovulation in AC were associated with patients who were older and those with a lower AMH value, a higher basal FSH value, and a lower starting oestrogen dose. When attempting to avoid follicular growth, we recommend that a higher exogenous dose of oestrogen is administered from the early phase of the menstrual cycle. Furthermore, our research demonstrates an increased implantation potential after the spontaneous follicular growth and ovulation event in AC-FET cycles. Cancellation of such cycles is not necessary. With careful monitoring and informed decisions, many valuable cycles could be saved.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' roles

Y.S. participated in the study design and drafted the article. H.J. performed the analysis and wrote the manuscript. W.J. participated in the acquisition and analysis of data. L.X., J.L., C.Z., M.Z. and S.C. reviewed the final article and made appropriate corrections and suggestions to improve it. X.L. and R.S. are corresponding authors and they participated in the study design, did the final proof reading and confirmed the final version. All authors approve the version to be published.

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Conflict of interest

The authors declare that they have no competing interests.

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