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Administration of depot GnRH agonist prior to programmed frozen-thawed embryo transfer does not improve the live birth rate in ovulatory women

A large, multi-center retrospective study

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Abstract

Despite that gonadotropin-releasing hormone (GnRH) agonist pretreatment has been widely used before programmed frozen-thawed transfer (FET), its impact on live birth rates in ovulatory women remains uncertain. In the present study, we aim to determine if GnRH agonists pretreatment before FET improves live birth rates in women undergoing in vitro fertilization with FET. Programmed FET cycles conducted in four infertility centers were retrospectively collected and reviewed for eligibility from January 2016 and December 2017. Patient's demographics, ovarian stimulation parameters, and pregnancy outcomes were compared between those given GnRH agonist pretreatment versus no pretreatment in ovulatory women undergoing FET cycles. A total of 6397 programmed cycles were screened for eligibility, of which 5049 cycles were included in the study for analysis. Compared with the group of no GnRH agonist pretreatment (n = 4143), women in the GnRH agonist group (n = 906) were older (33.0 vs 34.0, $P < .001$), had a higher proportion of subjects with previous transfer attempts and had a higher number of embryos transferred. After controlling for confounders, the logistic regression results showed that GnRH agonist pretreatment did not increase the odds of both clinical pregnancy (OR 0.92, 95% CI [0.70–1.20]), ongoing pregnancy (OR 0.91, 95% CI [0.69–1.19]) and live birth rates (OR 0.84, 95% CI [0.64–1.10]). However, when restricted to women who had no previous transfer attempts, women in the GnRH pretreatment group had lower odds of achieving live birth (OR 0.49, 95% CI [0.30–0.79]). Sensitivity analysis performed in patients with male factor infertility causes showed GnRH agonist pretreated group had lower live birth rates compared to no GnRH agonist pretreatment group (OR = 0.65, 95% CI [0.43–0.97]). Our findings suggested that GnRH agonist pretreatment does not bring additional benefits in live birth rate improvements for ovulatory women undergoing FET cycles. Therefore, the pros of using GnRH agonist to reduce premature ovulation should be weighed against the cons of prolonged time to pregnancy, discomforts resulting from pituitary suppression, and increased medical costs associated with GnRH agonist use.

Abbreviations: ER = endometrial receptivity, FET = frozen-thawed transfer, FSH = follicle-stimulating hormone, GnRH = gonadotropin-releasing hormone, HRT = hormone replacement therapy, ICSI = intracytoplasmic sperm injection, IVF = in vitro fertilization, LH = luteinizing hormone, RCT = randomized controlled trials.

Keywords: endometrial preparation, frozen embryo transfer, GnRH agonist, hormone replacement treatment

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The raw data supporting the conclusions of this article will be made available by the authors based on reasonable request.

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institution and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The Research Ethics Committees of the following centers have confirmed that no ethical approval is required as this is an observational study. The centers are The People's Hospital of Guangxi Zhuang Autonomous Region, Women and Children's Hospital of Guangdong Province, Family Planning Special Hospital of Guangdong Province, and Qinzhou Maternal and Child Health Care Hospital. Informed consent was obtained from all individual participants included in the study.

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1. Introduction

The number of frozen-thawed cycles has been increasing steadily over the recent years. One of the main reasons driving this persistent rise is the significant improvement of embryo cryopreservation techniques, resulting in comparable pregnancy outcomes between fresh and frozen-thawed cycles. Despite a significantly large number of frozen-thawed transfers (FET) been already performed in many infertility centers, the best regime of endometrial preparation before thawing and transferring is still under debate.

In general, the endometrium of FET is prepared by three approaches: natural cycles, stimulated cycles, and hormone replacement therapy (HRT) cycles. Natural cycles and stimulated cycles can be pragmatically difficult, requiring close monitoring of luteinizing hormone (LH) to time embryo transfer with ovulation. By contrast, the HRT approach is preferred by many clinicians as it allows scheduling embryo transfer flexibly and obviates the need for repetitive hormone checkups; nevertheless, such an approach is associated with a risk of spontaneous ovulation. Specifically, this endometrial preparation regime coincides with the transfer of warmed embryos into receptive endometrium by administrating estrogen and progesterone sequentially; as a result, the increasing serum estrogen level may induce the LH surge, also known as estrogen positive feedback. As a result, this early LH elevation often leads to premature progesterone rise and early opening of the implantation window and therefore, disturbing the synchronization of embryo development with the endometrium. To prevent unexpected ovulation, gonadotropin-releasing hormone (GnRH) agonist pretreatment prior to estrogen administration has been widely used to suppress the endogenous LH levels and therefore to reduce the cycle cancelation risk in FET cycles. Using such a pretreatment also has the added benefit of reducing the anxiety of patients for fearing cycle cancelation.

Despite these advantages, the clinical efficacy of GnRH agonist pretreatment for ovulatory women undergoing programmed FET cycles remains uncertain. Although there have been multiple randomized controlled trials (RCTs) comparing GnRH agonist pretreatment versus conventional endometrium preparation in women undergoing artificial FET cycles, the Cochrane meta-analysis published in 2020[1] graded the quality of the evidence to be low. Among the nine RCT studies included in the meta-analysis, only one study reported live birth as the outcome[2]; however, this study suffers from a high selection bias, hence compromising its reliability. Furthermore, the majority of observational studies have been focusing on infertile women without distinguishing the presence of ovulatory disorders, but it remains questionable whether the GnRH agonist findings from this heterogenous infertile population are directly applicable to ovulatory women.

In light of the low quality of existing evidence and the paucity of data using live birth as an outcome, our study sought to evaluate the use of GnRH agonist pretreatment's impact on live birth rates in ovulatory women undergoing HRT-FET cycles.

2. Material and Methods

2.1. Subjects

This is a multi-center retrospective study. Potentially eligible patients were identified by screening the electronic medical records from four reproductive centers across two provinces in southern China: the People's Hospital of Guangxi Zhuang Autonomous Region, the Women and Children's Hospital of Guangdong Province, the Family Planning Special Hospital of Guangdong Province, and Qinzhou Maternal and Child Health Care Hospital. All women underwent autologous in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), embryos vitrification and warming, programmed FET cycles between January 2016 and December 2017, regardless of the number of cycles they previously underwent. Ovulatory women were those who had regular menstrual cycles and consistently presented with ovulation for two consecutive months detected by ultrasound. Ovulation is defined as follicles over 16 mm detected by vaginal ultrasound and disappearing the next day.

The exclusion criteria are[1] those whose FET cycles were canceled due to no viable embryos after thawing[2]; FET cycles canceled because of women's endometrium abnormalities including endometrial polyps, adhesions or endometrial fluid accumulation on the day of embryo transfer[3]; cycles of women who had a fever or abdominal pain on the day of embryo transfer that her attending doctor considered not suitable for the procedure (Supplementary 4, http://links.lww.com/MD/H528). The Research Ethics Committees of each study site have confirmed that no ethical approval is required as this is an observational study.

2.2. Stimulation protocol

The controlled ovarian stimulation protocol used in this study were either the depot agonist protocol, the long agonist protocol, or the antagonist protocol, based on the clinical routine of each study site. Fourteen days before the menstrual cycle, women using the agonist protocol received either the depot GnRH agonist triptorelin at a dose of 1.875 mg or 1.25 mg injected once, or they were given triptorelin 0.1 mg injected daily until the triggering day. Two weeks later, transvaginal ultrasound was performed and serum hormone levels including follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were tested to confirm if the ovaries were quiescent and endometrium was thin (<5 mm). Stimulation with gonadotropins was initiated on the same day, with the starting dose between 150 and 300 IU, according to the patient's body mass index, age, and ovarian reserve. For the GnRH antagonist protocol, the starting dose of gonadotropin was the same as the agonist protocol. When the leading follicles measured 12 mm or above, Cetrorelix (Cetrotide; Merck Serono, Darmstadt, Germany) 0.25 mg was injected daily until the triggering day. When a minimum of two follicles' size reaching to 18 mm or over, urinary human chorionic gonadotropin (Lidebao, Livzon Pharm, Zhuhai, China) 10,000 IU was used for triggering. After 36 to 38 hours of triggering, oocytes were retrieved under the guidance of transvaginal ultrasound.

2.3. IVF and laboratory procedures

Retrieved oocytes were fertilized by IVF unless ICSI was indicated. The indications for using ICSI were described elsewhere.[3] Fertilization was assessed after 16 to18 hours of insemination based on the presence of two distinct pronuclei and two polar bodies. Embryos were then cultured to the cleavage stage. Those cleavage-stage embryos with 6 or more cells and less or equal to 20% of fragmentation were labeled as good quality embryos and were eligible for either transfer or cryopreservation. After 5 days of culture, embryos were graded and selected based on their morphology using the Gardner criteria.[4] Those embryos who failed to meet the criteria for good quality cleavage embryos were cultured to blastocyst stage; embryos scored with ≥ 4BC based on the Gardner criteria were considered for transfer or cryopreservation.

2.4. Endometrium preparation

For women undergoing GnRH agonist prior to the programmed cycle, 3.75 mg of triptorelin (Decapeptyl, Ferring, St-Prex, Switzerland) was administrated intramuscular on the second or the third day of the menstrual cycle before the FET cycle. After 28 days of the injection, estradiol valerate (Progynova, Bayer, Leverkusen, Germany) 6 mg daily was administrated orally

starting from the second or the third of the menstrual cycle. Since then, transvaginal ultrasound was performed and serum progesterone was tested once a week to evaluate the thickness of the endometrium and to rule out premature ovulation. When the thickness of the endometrium reaches 7 mm or above and patients' serum progesterone level measuring <1.5 ng/mL, vaginal progesterone (Crinone, Merck Serono) 90 mg once a day or 60 mg progesterone daily intramuscular injection (progesterone injection, Xianju Pharma, Taizhou, China) was administrated. The decision of choosing which progesterone route was entirely based on the patients' preference. If the pregnancy was established, estradiol valerate dosage was tapered off while progesterone supplementation was sustained until 10 to 12 weeks of gestation. For women with no GnRH agonist pretreatment, estradiol and progesterone were given as the preceding dose described above, the same as patients undergoing GnRH agonist pretreatment. There was no medical indication for GnRH agonist pretreatment and the decision of whether to use the pretreatment was entirely dependent on the physicians' and patients' preferences for better scheduling and minimizing the cycle cancelation risk.

For patients undergoing cleavage-stage embryos transfers, the transfer was performed on the fourth day of progesterone supplementation. Up to three cleavage embryos were allowed to transfer for each patient. For women who had blastocysts to transfer, the transfer was scheduled on the sixth day of the progesterone administration. A maximal of two blastocysts could be transferred for each patient. The modified Cryotop® method was used as the blastocyst warming method.[5] The embryo transfer procedure was performed under the guidance of transabdominal ultrasound.

2.5. Outcome measurements

The independent variable of interest was the GnRH agonist pretreatment prior to the programmed cycle, defined as whether the GnRH agonist before the initiation of estradiol and progesterone during HRT FET cycles was used. The primary outcome used in this study was live birth, defined as the live birth of an infant exceeding 24 weeks of gestation. The secondary outcomes included biochemical pregnancy, defined as serum β-hCG levels measuring ≥5 IU/L at 14 days after embryo transfer. Implantation rate was defined by the number of gestation sacs divided by the number of embryos transferred; early pregnancy loss is defined as a clinical pregnancy loss before 22 completed weeks of gestation[6]; ongoing pregnancy rate was defined as the number of viable intrauterine pregnancy of at least 12 weeks of gestation per number of FET cycles.[7]

2.6. Statistical analysis

For descriptive analysis of demographic and cycle characteristics, continuous variables were presented as the median and interquartile range (25–75% percentile), while categorical variables were expressed as frequency (n) and percentages (%). The distribution of normality was tested using the Shapiro–Wilk test. Baseline characteristics were compared between the study and the control group using Kruskal–Wallis *H* test, Pearson χ^2, or Fisher's exact test, where appropriate. The following variables were considered as potential confounders, including women's age, IVF centers, infertility causes, history of uterine abnormalities, number of high-quality embryos transferred, embryo stages during transfer, the times of previous transferred cycles, serum progesterone levels on the day of progesterone initiation, and serum luteinizing hormones levels on the day of progesterone initiation, and endometrium thickness on the triggering day. The effects of GnRH agonist pretreatment on pregnancy outcomes were calculated by using a binary logistic regression model while taking account of confounders. The crude odds ratios (ORs) and adjusted OR with 95% confidence intervals (95% CIs) were calculated to estimate the degree of association. A two-sided level of .05 was considered statistically significant. SPSS (SPSS Inc., Chicago, IL) version 16.0 was used as statistical analysis software.

3. Results

3.1. Screening

A total of 6397 programmed FET cycles were reviewed for screening, of which 1348 cycles were excluded for the following reasons: 1218 had irregular menstrual cycles; 12 canceled the embryo transfer because of no viable embryos after thawing; 15 cycles canceled due to the patients' personal reasons; 58 cycles lost to follow up to ascertain their pregnancy status. As a result, 5049 FET cycles were included in the final analysis, of which 4143 cycles were those treated with conventional artificial endometrium preparation whereas 906 cycles were given GnRH agonist pretreatment plus conventional artificial endometrium preparation.

3.2. Demographic and cycle characteristics comparison

As is shown in Table 1, compared with no GnRH agonist pretreatment, cycles that were pretreated with GnRH agonist were women of older age (33.0 vs 34.0, $P < .001$). Women in the GnRH pretreatment group had a significantly lower proportion of male factor infertility (18.6% vs 12.3%) and diminished ovarian reserve related infertility (4.0% vs 5.5%), but tubal factor was the major indication for IVF in both groups (62.8% vs 62.0%). GnRH agonist-pretreated group also experienced more embryo transfer attempts previously and had a higher proportion of fewer than two embryos transferred (embryo transferred two or more in controls and cases group: 16.3% vs 25.3% $P < .001$). Although the GnRH agonist pretreatment group had a higher dose of estradiol during endometrium preparation (98 vs 108, $P < .001$), their serum estradiol on the progesterone initiation day was comparable between the groups (239.5 vs 235.7, $P < .639$). The progesterone and luteinizing hormone levels on the embryo transfer day were significantly lower in the GnRH agonist pretreated group (0.37 vs 0.23, $P < .001$); the GnRH agonist pretreated group also had significantly thicker endometrium compared to no GnRH agonist pretreatment group (9.0 vs 10.0 $P < .001$). There was no significant difference in the body mass index of women at transfer, fertilization method, serum basal FSH, and days of estradiol supplementation between the two groups.

3.3. Pregnancy outcomes

Compared to no GnRH agonist pretreated group, both ongoing pregnancy rates (47.3% vs 42.7%, $P = .012$) and live birth rates (44.7% vs 39.8%, $P = .008$) were significantly lower in the GnRH agonist pretreatment group (Table 2). However, after controlling for confounders, GnRH agonist pretreatment did not modify the odds of achieving ongoing pregnancy (adjusted OR, 95% CI 0.91 [0.69–1.19]) and live birth (adjusted OR 0.84, 95% CI [0.64–1.10]). The confounders were the age of women at transfer, IVF centers, infertility causes, history of endometrial abnormalities, number of high-quality embryos transferred, blastocyst or cleavage embryos, the number of previous embryo transfer attempts in FET, serum progesterone levels and serum luteinizing hormones levels on the day of progesterone initiation and endometrium thickness on the triggering day (Table 3).

3.4. Sensitivity analysis results

Considering the previous embryo transfer attempts may have confounded the result, the sensitivity analysis based on this

Table 1

A comparison of baseline demographic and cycle characteristics according to whether patients received GnRH agonist prior to the artificial cycle of frozen embryo transfer.

Variables	No GnRH agonist pretreatment (n = 4143)	GnRH agonist pretreatment (n = 906)	*P* value
Age at embryo transfer (yr)	33.0 (29–37)	34.0 (30–38)	<.001
<30	27.5% (1141)	20.1% (182)	<.001
30–35	33.8% (1402)	33.7% (305)	.919
35–40	23.3% (967)	27.3% (247)	.012
≥40	15.3% (633)	19.0% (172)	.006
Body mass index (kg/m^2)	21.3 (19.5–23.4)	21.2 (19.5–23.2)	.580
Serum basal FSH (IU/L) (mIU/mL)	6.7 (5.6–7.9)	6.5 (5.4–7.8)	.160
Causes of infertility			
Tubal factor	62.8% (2603)	62.0% (561)	<.001
Diminished ovarian reserve	4.0% (164)	5.5% (50)	
PCOS	2.4% (101)	0.8% (7)	
Male factor	18.6% (772)	12.3% (112)	
Endometriosis	2.9% (122)	6.0% (54)	
Unexplained infertility	9.2% (381)	13.4% (121)	
Fertilization method			
IVF	73.0% (3023)	77.0% (698)	.012
ICSI	27.0% (1120)	23.0% (208)	
Previous attempts in embryo transfers			
0	50.4% (2087)	40.1% (363)	<.001
1	33.4% (1382)	34.7% (314)	
2 or more	16.3% (674)	25.3% (229)	
The number of high-quality embryos transferred			
1	11.5% (476)	14.7% (133)	.003
2	40.3% (1670)	42.4% (384)	
3	48.2% (1997)	42.9% (389)	
Estradiol supplementation length (d)	12.8 (12–14)	12.8 (12–14.4)	.142
Total estrogen dose (mg)	98 (83–117)	108 (90–122)	<.001
Serum hormone levels on the day of progesterone initiation			
Estrogen levels (pg/mL)	239.5 (171.8–399.1)	235.7 (171.2–432.7)	<.639
Progesterone levels (ng/mL)	0.37 (0.21–0.56)	0.23 (0.09–0.39)	<.001
Luteinizing hormone levels (mIU/mL)	11.7 (7.1–18.3)	0.64 (0.35–1.23)	<.001
The endometrial thickness on the triggering day (mm)	9.0 (8–10)	10.0 (8.6–11.1)	<.001

Diminished ovarian reserve is defined as those with antral follicle count <7 and anti-mullerian hormone <1.1 ng/mL. Data are expressed as median (Q1, Q3) or percentage of patients (n).
FSH = follicle-stimulating hormone, GnRH-a = gonadotropin-releasing hormone agonist, PCOS = polycystic ovarian syndrome.

Table 2

The comparison of pregnancy outcomes between no GnRH agonist pretreatment group and GnRH-a pretreated group.

	No GnRH agonist pretreatment (n = 4143)	GnRH agonist pretreatment (n = 906)	*P* value
Implantation rate	41.1% (3103/7552)	38.6% (632/1636)	.067
Biochemical pregnancy rate	61.2% (1550/2534)	61.8% (560/906)	.720
Clinical pregnancy rate	55.0% (2279/4143)	51.7% (468/906)	.066
Multiple pregnancy rate	36.9% (841/2279)	35.5% (166/468)	.840
Early pregnancy loss rate	16.9% (386/2279)	20.5% (96/468)	.064
Ongoing pregnancy rate	47.3% (1960/4143)	42.7% (387/906)	.012
Live birth rate	44.7% (1852/4143)	39.8% (361/906)	.008

Data are expressed as the percentage of patients (n).
GnRH-a = gonadotropin-releasing hormone agonist.

factor was conducted accordingly. The results showed that for patients who underwent their first FET cycles, the GnRH agonist pretreatment group resulted in significantly lower clinical pregnancy rates (59.2% vs 49.3%, $P < .001$), but no statistical difference was found in adjusted logistic regression results (OR 0.69, 95% CI [0.44–1.09]). However, both the univariate (49.5% vs 34.7%, $P < .001$) and adjusted logistic regression analysis (OR 0.49, 95% CI [0.30–0.79]) showed the consistent findings that GnRH agonist pretreatment had lower live birth rates compared with no GnRH agonist pretreatment, as is shown in Table 4.

Given that age is a confounder, we further stratified the population based on the patients' age, and the results revealed that no significant statistical difference was found on the clinical pregnancy rates and live birth rates between the two groups in each age stratum (Supplementary Table 1, http://links.lww.com/MD/H525). Additionally, we also restricted our analysis to standard patients, defined as those who underwent first FET cycle, were younger than 35 years old, with no history of endometrium abnormalities (including endometrial polyps, intrauterine adhesions, and intramuscular fibroids), adenomyosis, endometriosis, scarred uterus, and those had at least one high-quality embryo transfer. Similarly, ongoing pregnancy and live birth rates did not reach a significant statistical difference between the two groups (Supplementary Table 2, http://links.lww.com/MD/H526).

When stratifying patients based on their infertility causes, there was no statistical difference in clinical pregnancy rates and live birth rates between the study and the control group in patients with tubal factors, diminished ovarian reserve, endometriosis and unexplained infertility. However, patients with male factor infertility pretreated with GnRH agonist had lower live birth rates compared with no GnRH agonist pretreatment (OR = 0.65, 95% CI [0.43–0.97]) (Supplementary Table 3, http://links.lww.com/MD/H527).

Table 3

The odds of achieving pregnancy and experiencing early pregnancy loss after frozen embryo transfer (FET) according to whether GnRH agonist pretreatment is received before the programmed cycles.

	Crude odds ratio (95% CI)	Adjusted odds ratio (95% CI)*
Biochemical pregnancy	1.02 (0.88–1.19)	0.85 (0.65–1.12)
Clinical pregnancy	0.87 (0.75–1.01)	0.92 (0.70–1.20)
Early pregnancy loss	1.29 (0.98–1.68)	1.04 (0.66–1.65)
Ongoing pregnancy	0.83 (0.72–0.96)	0.91 (0.69–1.19)
Live birth	0.83 (0.71–0.96)	0.84 (0.64–1.10)

The reference group is programmed FET cycles without GnRH agonist pretreatment.
CI = confidence interval, GnRH = gonadotropin-releasing hormone agonist, IVF = in vitro fertilization.
*The model was adjusted for age of women at transfer, IVF centers, infertility causes, history of endometrial abnormalities, number of high-quality embryos transferred, blastocyst or cleavage embryos, the number of previous embryo transfer attempts in FET, serum progesterone levels and serum luteinizing hormones levels on the day of progesterone initiation and endometrium thickness on the triggering day.

Table 4

A sensitivity analysis of the pregnancy outcomes between women undergoing programmed FET cycles with and without GnRH agonist pretreatment.

	No GnRH agonist pretreatment (n = 4143)	GnRH agonist pretreatment (n = 906)	*P* value	OR (95% CI)*
Previous embryo transfer times	Clinical pregnancy rate			
0	59.2% (1236/2087)	49.3% (179/363)	<.001	0.69(0.44–1.09)
1	53.0% (733/1382)	53.5% (168/314)	.880	1.49(0.94–2.37)
2	46.0% (310/674)	52.8% (121/229)	.073	0.85(0.49–1.47)
Previous embryo transfer times	Live birth rate			
0	49.5% (1034/2087)	34.7% (126/363)	<.001	0.49(0.30–0.79)
1	41.6% (575/1382)	44.9% (141/314)	.290	1.56(0.98–2.46)
2	36.2% (243/674)	41.0% (94/229)	.180	0.85(0.49–1.46)

FET = frozen-thawed transfer, GnRH-a = Gonadotropin-releasing hormone agonist, IVF = in vitro fertilization.
*The model was adjusted for age of women at transfer, IVF centers, infertility causes, history of endometrial abnormalities, number of high-quality embryos transferred, blastocyst or cleavage embryos, serum progesterone levels and serum luteinizing hormones levels on the day of progesterone initiation and endometrium thickness on the triggering day. The reference group is programmed frozen transfer cycles without GnRH agonist pretreatment.

4. Discussion

The current study found no significant statistical difference in the live birth rate in women undergoing FET cycles following GnRH agonist pretreatment compared to no pretreatment before FET cycles. This lack of association persisted in sensitivity analysis including those in different age stratum; furthermore, for those who underwent their first FET cycles or patients with male factor infertility, GnRH agonist pretreatment was associated with lower live birth rates in comparison of those who did not receive GnRH agonist pretreatment. To our knowledge, this is the largest observational study involving multi-centers thus far evaluating the impact of GnRH agonist pretreatment on pregnancy outcome among ovulatory women undergoing artificial FET. Our results are particularly relevant in this era where an increasing number of embryos are undergoing freezing and thawing, and the programmed cycle remains a major endometrium preparation regime for FET.

In line with our study, the absent benefits of GnRH agonists use before programmed FET was also reported by other research groups.[8,9] For example, Dal Prato et al[8] conducted an RCT allocating 146 patients in the GnRH agonist pretreatment group and 150 patients in no GnRH agonist pretreatment. The results showed that the clinical pregnancy rates were 19.7% in GnRH agonist-pretreated women compared to 24.1% in women without GnRH agonist pretreatment group, but no statistical difference was observed; however, it was the oldest of the studies, reported two decades ago in 2002. More recently, another RCT by Movahedi et al[10] involved 100 women who were randomly assigned to buserelin pretreatment (n = 60) versus no pretreatment (n = 40) when undergoing artificial FET; the clinical pregnancy rates in the GnRH agonist pretreatment group and conventional treatment group was 15.0% and 17.5%, respectively, and no significant statistical difference was revealed. Our results reported here expand on this work with respect to sample size and outcomes of interest. However, contrary to our findings, a study by El-Toukhy et al[2] showed GnRH agonist-pretreated women had significantly higher live birth rates compared with no GnRH agonist pretreated women (20% vs 8.5%, OR 2.9, 95% CI [1.2–8], *P* = .01). One of the reasons for the discrepancy between our study and the study by EI-Toukhy et al is that in their study, the average length of estrogen was nearly three weeks in both groups (20.7 days vs 21 days, *P* = .7), whereas our study only had an average of 12 days of estrogen administration prior to progesterone use; therefore, the prolonged use of estrogen in the study by EI-Toukhy et al may have had resulted in under-detected premature progesterone rise in women without pituitary suppression before programmed FET, resulting in embryo-endometrium asynchronization and therefore poor pregnancy outcome. Another reason contributing to the disagreement is the differences in forms and routes of GnRH agonist; the agonist used by EI-Toukhy et al were buserelin nasal spray administrated daily whereas our study used depot triptorelin that only requires one intramuscular injection.

In our database, there were <10% of endometriosis patients in the GnRH agonist pretreated group in the frozen-thawed embryo transfer cycles. There are two main reasons for the small proportion of endometriosis in the GnRH agonist treated group. First, although the GnRH agonist pretreatment was traditionally used in endometriosis, this has not become a routine of practice in infertility centers in China. In contrast, GnRH agonist pretreatment before FET cycles was also widely used in various populations including women with recurrent implantation failure,[11] elderly patients,[12] and polycystic ovary syndrome[13]; all the studies cited were conducted in the Chinese population. Second, reducing the time to pregnancy is an important factor to consider when making clinical decisions; maximizing the chances of transferring the embryo in the fresh cycle has been the goal for clinicians in all of the four centers in our study. As a result, nearly half of the patients with endometriosis had undergone fresh embryo transfer, leaving a truncated number of endometriosis patients undergoing frozen-thawed embryo transfer cycles.

The molecular mechanisms underlying the GnRH agonist use on pregnancy establishment and maintenance remain unclear. Despite that there is evidence supporting the administration GnRH agonists improves the endometrial receptivity (ER) via

up-regulating ER mediators including homeobox A10, myeloid ecotropic viral integration site 1 and leukemia inhibitory factor, such a beneficial effect of GnRH agonist on implantation window is merely hypothetical.[14,15] This uncertainty is because samples of ER evaluation were most often derived from mice or women who administrated the long agonist protocol but canceled their cycle because of the high risk of ovarian hyperstimulation syndrome. As a result, the compromised ER may have been a result of ovarian hyperstimulation syndrome, but not the GnRH agonists use.[15,16] Based on our results, we speculate that for ovulatory women who had normal pituitary–ovarian axis function, pretreating patients using GnRH agonist prior to FET may not improve endometrium receptivity; rather, the pituitary suppression drug may have a remnant adverse impact on sustaining the pregnancy in a certain population. As was seen in our study, when we restricted the population to those who underwent their first FET cycles, the clinical pregnancy rates were similar between the groups but fewer women achieved live birth in the GnRH agonist-treated group.

The major strength of our study is that as a large, multi-center study, we included women from four IVF centers located in the different geographical areas of China, thereby significantly improving the external validity of our results. The large sample size also allowed us to have adequate statistical power to detect a statistically significant effect of GnRH agonist pretreatment with respect to pregnancy outcomes. Furthermore, clinically relevant outcomes, such as clinical pregnancy and live birth, were reported in the present study. To exclude the influence of potential confounders, we performed multiple sensitivity analyses to investigate how the variation in age, the number of previous transfer attempts and the infertility causes would affect the pregnancy outcomes between the groups, hence increasing the internal accuracy of our results.

However, our study has several limitations. First, due to the nature of the retrospective study design, GnRH agonist pretreatment was not randomly assigned and hence may introduce selection bias. Nevertheless, compared to women who received GnRH agonist pretreatment, those women without pretreatment were younger and had a lower proportion of women with diminished ovarian reserve. Given their age and ovarian reserve advantage, women with GnRH agonist pretreatment should be expected to be seen a better pregnancy outcome; yet, our results showed GnRH agonist had no additional benefits in improving pregnancy outcomes. On the contrary, when restricted population to those who had no embryo transfer attempts, an adverse impact of GnRH agonist on live birth rates was observed. Therefore, the differences in pregnancy outcomes are unlikely caused by selection bias alone. Second, the unit of analysis used in the present study was the number of FET cycles, rather than the number of women who underwent FET. Utilizing such a unit of analysis may overestimate the true treatment effect of GnRH agonist. This overestimation is because women were administrated conventional HRT in their first FET cycle but were given GnRH agonist pretreatment in the next cycle. In this scenario, a single woman was included twice, both in the study and the control group. However, we believe this scenario reflects the true clinical practice where a single patient often switches from one endometrium preparation to another when her first FET cycle failed. To rule out such a misleading effect, we did a sensitivity analysis restricting the population to those who had no previous transfer attempts. Third, cycle cancelations due to premature ovulation were not analyzed as the data were not available to collect due to technical difficulties in data access. Indeed, our results showed that women pretreated with GnRH agonist had lower serum LH and progesterone levels compared with those without agonist pretreatment, but their premature levels were considerably below 1.5 ng/mL, suggesting the premature ovulation did not occur in both groups of women included in the study. Moreover, although it would have been meaningful to include cycle cancelation rate in our study to determine the advantage of GnRH agonist use in reducing cycle cancelation, a recent Cochrane review found no evidence of a difference between the two groups in cycle cancelation rate (OR 2.73, 95% CI [0.79–9.38], 3 RCTs, n = 636).[17]

5. Conclusion

In conclusion, our results suggest that for women undergoing artificial FET cycles, skipping GnRH agonist pretreatment may be superior to its use for ovulatory women undergoing artificial FET cycles. The live birth rate was compromised in women undergoing their first frozen embryo transfer and patients with male factor infertility causes. We conclude that despite that GnRH agonist may have benefits in reducing premature ovulation, the pros and cons of reducing premature ovulation should be weighed with regard to reduced live birth rate, prolonged time to pregnancy, discomforts resulting from pituitary suppression and increased medical costs associated with GnRH agonist use.

Author contributions

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