Endometrial Preparation for Frozen–Thawed Embryo Transfer With or Without Pretreatment With Gonadotropin-Releasing Hormone Agonist in Regular Menstrual Cycles: Results of a Retrospective Cohort Study

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Abstract

Objects

To compare the treatment outcomes of FET in hormonal replacement treatment (HRT) protocol with and without long-acting gonadotropin-releasing hormone agonist (GnRHa) pretreatment in patients with regular menstrual cycles.

Methods

A total of 5049 patients from three centers were recruited in this retrospective study. The study population was divided into two groups. In HRT with GnRHa group, endometrial preparation with supplement estrogen 6mg daily initiated following down-regulation of long-acting GnRH agonist. In HRT group, the same dose of estrogen administration only for two weeks. Live birth rates were the primary outcomes measured in both groups.

Results

There were no differences in implantation rate, β-HCG positive rate, clinical pregnancy rate, and early miscarriage rate between the two groups. The ongoing pregnancy rate and the live birth rate were higher in HRT group compared to the agonist group (47.3% vs 42.7% and 44.7% vs 39.8%, respectively). However, the co-treatment with GnRH agonist in HRT protocol was not associated with clinical pregnancy rate (OR 0.94; 95% CI 0.78 to 1.12) and live birth rate (OR 0.82; 95% CI 0.71 to 1.02) after logistic regression analysis. In the first FET cycle, patients in HRT group achieved higher clinical pregnancy rate and live birth rate. In addition, there was no differences in clinical pregnancy rate and live birth rate between the two groups in standard patients.

Conclusions

The HRT protocol for FET seems to be as effective as the HRT protocol involving preliminary pituitary suppression with GnRH agonist in regular menstrual cycles.

Introduction

In recent years, the number of frozen-thawed embryo transfer (FET) cycles has significantly increased due to improvements in laboratory techniques and the availability of surplus embryos generated by in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) cycles[1,2]. FET reduces embryos wastage and increases the chances of cumulative pregnancy after one cycle of ovarian hyper-stimulation and oocyte retrieval in IVF/ICSI[3]. Many studies have concluded that FET reduces the risk of preterm delivery and low birth weight[4]. However, the best regimen for endometrium preparation in ovulatory women is still a matter of debate.

The synchronization of endometrium and embryo development are critical to embryo implantation and can be achieved by various methods[5]. The simplest regimen for endometrium preparation in FET is natural cycle (NC). The point of embryo–endometrium synchronization assessment in NC is the ovulation of the dominant follicle, which can be monitored either by serial serum LH level assessing until an LH peak is observed (true NC with spontaneous ovulation) or by triggering hCG (modified NC, hCG is applied to trigger final ovulation)[6]. However, although the advantage of NC is free of estrogen administration, it requires more frequent visits to the hospital, less flexibility, and high risk of cycle cancellation. Thus its application is limited in clinic practice[5,7].

Apart from natural cycle, hormonal replacement treatment (HRT) is a frequently alternative regimen because of its flexible schedule and less monitoring. In HRT cycle, estrogen is administered for two weeks to cause endometrial proliferation and suppress dominant follicle growth. However, HRT cannot definitely suppress pituitary, so that follicles growth and ovulation escape may happen occasionally. To overcome this disadvantage, pre-treatment with GnRH agonist is added to the HRT protocol for better pituitary down-regulation and spontaneous ovulation prevention[5,8]

Although several randomized controlled trials (RCT) have investigated the benefits of GnRH agonists in HRT cycle, but their conclusions were inconsistent. In one RCT, HRT with GnRH agonist pre-treatment was reported to achieve significant higher pregnancy rate and live birth rate than HRT only[9]. The result contradicted other RCTs, which failed to demonstrate any beneficial use of the GnRH agonist in terms of clinical pregnancy rate[10-13]. However, those RCTs have several limitations. Firstly, Their sample sizes are small, so that statistical power may be inadequate. Secondly, only one study considered live birth rates as the primary outcome, and other outcomes such as ongoing pregnancy rates and miscarriage rates were rarely reported. Two meta-analyses on this subject had reported the considerable heterogeneity of the above RCTs. They also expressed concern about the quality of the evidence due to the small sample size, poor reporting of study methods, and failure to report critical clinical outcomes in those RCTs[14,15]. Therefore, the conclusion of the RCTs should be interpreted with caution. Currently additional studies on this topic with a large sample size and a good design are still required.

In view of the present conflicting clinical outcomes, and the lack of strong evidence, this large multicentric retrospective cohort study aimed to compare the treatment outcomes of FET in HRT protocol with and without GnRH agonist.

Methods

This study is a retrospective cohort analysis aiming to compare two protocols of endometrial preparation for FET cycles (HRT vs HRT with GnRH agonist) in patients with regular menstrual cycles. Patients were from three different reproductive medical centers (The People's Hospital of Guangxi Zhuang Autonomous Region, Women and Children's Hospital of Guangdong Province, and Family Planning Special Hospital of Guangdong Province) from January 2016 to December 2017. The institutional review boards of the three

hospitals approved the study. However, we were unable to obtain written informed consent from participants due to the retrospective study design.

Patients

Patients were recruited from the three centers from January 2016 to December 2017. The criteria for exclusion were as follows: 1) irregular menstruation, 2) FET cycles were canceled due to embryo not available post thaw. 3) FET cycles were canceled due to patients' physical causes (e.g fever, abdominal pain), 4) serious hysteromyoma or intrauterine adhesions, and 5) lack of clinical outcomes due to loss of follow-up.

Endometrial preparation protocol

In general, patients with HRT protocol started to take oral estrogen (Progynova, Bayer, Germany, 6 mg daily.) on day 2-4 of the menstrual cycle. After estrogen supplement for 12-14 days, vaginal ultrasound scan and serum estradiol and progesterone measurements were performed for the monitoring of endometrial thickness and confirmation of follicle growth suppression. After the endometrial thickness exceeded 7 mm and serum progesterone less than 1.5ng/ml, exogenous progesterone supplement (vaginal gel 8% Crinone 90mg, daily, Merck Serono) was initiated, and frozen embryo replacement was scheduled. Cleavage-stage embryos were generally transferred on the fourth day of progesterone supplementation, whereas blastocysts were usually transferred on the sixth day. In another group, patients administered HRT with GnRH agonist received a dose of 3.75 mg of long-acting GnRH agonist (Diphereline, Ipsen Corporate, France) on the day 2-4 of the previous menstrual cycle, then followed by estrogen supplement 28 days later after the injection of GnRH agonist. The following HRT regimen was the same as HRT only group. The choice of protocol depends on the opinions of the physicians and the attitudes of the patients.

Embryo warming and transfer technique

Embryo warming was performed in accordance with the Vitrolife Rapid Warm protocol. In brief, warming was started by placing the vitrified embryos quickly into the equilibration solution. The embryos were allowed to fall from the device and sink to the bottom and then left for 10–30 seconds. The embryos were transferred to a dilution solution for 15 seconds, transferred to another dilution solution for 30 seconds, and washed with a solution without sucrose for 1 minute. Eventually, the embryos were transferred to a cleavage or blastocyst culture medium for another 4 hours of incubation. Then the surviving embryos were transferred into the uterus under ultrasound guidance. During the tansfer, patients were asked to present with a full bladder for uterus visualization by ultasound. Each embryo was loaded into a catheter tip and placed 1.0–2.0 cm below the apex of the uterine cavity, as determined by transabdominal ultrasound. In the entire procedure, the operation should be gentle to prevent the catheter to contact the uterine fundus.

Outcome measures

Pregnancy was first assessed by measuring serum β-hCG levels 14 days after FET. The clinical pregnancy rate referred to the number of the presence of at least one fetus with a heartbeat at 4 weeks after FET divided by the number of FET cycles. The implantation rate was calculated as the number of gestation sacs divided by the number of embryos transferred. Ongoing pregnancy rate was defined as the number of viable intrauterine pregnancy of at least 12 weeks duration divided by the number of FET cycles. Live birth rate referred to the number of deliveries that resulted in at least one live birth after FET divided by the number of FET cycles.

Statistical analysis

The data were analyzed by SPSS version16.0 for Windows (SPSS Inc., Chicago, IL, USA). Numeric variables were presented as median (quartile), and character variables were reported as a percentage (number). The median was compared using Kruskal-Wallis H test. The frequencies of categorical variables were compared using Pearson $\chi 2$ or Fisher's exact test, when appropriate. The binary logistic regression model was used to estimate the effect of GnRH agonist administration in artificial endometrial preparation for frozen-thawed embryo transfer on the clinical outcomes regarding relative covariates. $P<0.05$ was considered significant.

Results

A total of 6397 cycles were recruited, and 1,348 cycles were excluded for the following reasons: 1,218 patients with irregular menstruation; 12 cycles without transplantable embryos after thawing, 15 cycles canceled for personal reasons, 58 cycles without clinical outcomes. The final analysis was performed in 5049 cycles. The detail was present in Figure 1.

Cycles' baseline characteristics are presented in Table1. No significant difference was found between the two groups in BMI, basal serum FSH and type of embryo transferred. The median age was higher in the agonist group. The indications of fertility treatment in both groups were mainly tube factors, and the proportion of male factors and endometriosis in the two groups was different. Patients in agonist group more likely to have a history of FET. Most patients in both groups have at least one high-quality embryo transferred, but more cycles in agonist group can only transfer the poor quality embryos. The total estrogen days were similar in two groups, while the total estrogen dose was higher in agonist group. On the day of progesterone initiation, serum LH and serum P level were higher in HRT group, while the serum E2 were similar in the two groups. Additionally, the median endometrial thickness was higher in agonist group. (Shown in Table 1).

There were no differences in implantation rate, β-HCG positive rate, multiple pregnancy rate, early miscarriage rate, and clinical pregnancy rate between the two study groups. The ongoing pregnancy rate was higher in HRT group compared to the agonist group. Similarly, a higher live birth rate was observed in HRT group. (Shown in Table 2). The logistic analysis showed that the supplement of GnRH agonist was not associated with clinical outcomes including β-HCG-positive, clinical pregnancy, early miscarry,

ongoing pregnancy, and live birth rate after a variety of confounding factors controlled. (Shown in Table 3).

Given the significant difference in age and transplant time between the two groups, the data were further analyzed according to age cycle of transfer. The clinical pregnancy rate and live birth rate was reduced with increasing age, whereas no difference was observed between the two groups among different age groups (Shown in Supplement Table 1). In the first FET cycle, patients in HRT group appeared to have reached a higher clinical pregnancy rate. Conversely, the clinical pregnancy rate in the agonist group was higher in the patients undergoing their third or more transfer cycle. However, the difference was not statistically significant after confounding factors controlled. The live birth rate was significant higher in HRT group when only the first transfer cycle was considered, and the difference still observe in logistic regression analysis. In the third or more FET cycle, there was a higher live birth rate in the agonist group but the difference did not reach statistical significance. (Shown in Table 4).

In order to reduce possible selection bias, we screen out standard patients according to the following criteria: first FET cycle, younger than 35 years old, no history of uterine lesions(including endometrial polyps, intrauterine adhesions, uterine fibroids, adenomyosis, endometriosis, scarred uterus,etc.), at least one high-quality embryo transfer. Finally, a total of 2208 standard patients were selected. There were no differences in β-HCG positive rate, clinical pregnancy rate, ongoing pregnancy rate, early miscarriage rate and live birth rate between the two groups in standard patients. (Shown in Table 5)

Discussion

To the best of our knowledge, this is the largest retrospective cohort study aim to compare the treatment outcomes of FET in HRT protocol with and without GnRH agonist. Our data suggest that the artificial endometrium preparation using estrogen and progestin only for FET seems to be as effective as the protocol involving preliminary pituitary suppression with GnRH agonist.

An important consideration in implantation and development of pregnancy of FET cycles is the preparation of the endometrium prior to embryo transfer[16,17]. Although many studies have reviewed this issue, a consensus is lacking on the optimum method for endometrial preparation. Compared with the natural cycle, the use of GnRH agonist in hormonal replacement treatment protocol has several advantages: it can drastically reduce the cancellation rate and enable the flexibility of embryo transfer timing in accordance with the preferences of medical staff or patients, and can lessen the need for repetitive blood examinations and transvaginal ultrasound for endocrine and endometrial proliferation monitoring[5,18]. However, this protocol has disadvantages. The preparation is prolonged, the cost increases given the use of a GnRH agonist, the GnRHa can have side effects and may delay the resumption of spontaneous ovulation if FET fails[19]. A hormonal replacement treatment without the prior administration of GnRH agonist may be ideal for the endometrial preparation of FET cycles because it retains the advantages of low cancellation rate and flexibility in embryo transfer of artificial cycle; it

simultaneously simplifies and reduces the time and money consumption and may thus be more convenient and acceptable for patients if it achieves a similar clinical outcome.

The clinical pregnancy rate is a common outcome measured in comparative effectiveness research. Our results are in line with previously published prospective randomized studies[10-13]. In those studies, endometrial preparation for FET administered exclusively on steroids, appears to be as effective as the conventional regiment involving desensitization using GnRH agonist with regard to clinical pregnancy rates. However, this present study results obviously contrast with another randomized trial, which concluded that the supplement of GnRH agonist associated with a higher pregnancy rate and live birth rate[9]. In the latter study, only ultrasound was used for cycle progression monitoring and without ultrasound and/or endocrine monitoring of ovulation included. It is also worth noting that the average duration of the estrogen supplementation in that study is nearly three weeks, and that may have potentially induce a higher rate of ovulation in the patients without down-regulated as compared with other regimens with fix day (14 day) of estrogen administration[20]. Unobserved ovulation, and thus inappropriate timing of transfer, might explain the low clinical pregnancy rate of HRT group found in that study. A Cochrane review evaluated the comparison of 7 RCT and concluded that no evidence of a difference between the two groups in the clinical pregnancy rate[15]. Another meta-analysis also showed that neither of the two regiments had a significant advantage in terms of the clinical pregnancy rate[14]. However, both researchers acknowledge that considerable heterogeneity was detected. They also emphasized that the quality of the most evidence was low or very low due to small sample size, poor reporting of study methods and failure to report important clinical outcomes. So their conclusion should be interpreted with caution. Higher ongoing pregnancy rate and live birth rate were observed in HRT group in our study, but the difference did not reach statistically significant after adjustment for confounding factors by regression analysis. Ongoing pregnancy was only report in a single study and our result was consistent with theirs[13]. Only three studies considered live birth rate as outcome, and our results were consistent with our previous study focus on elderly patients and another retrospective study. In those two studies, the use of GnRH agonist in artificial endometrium cycles to increase live birth rates cannot be established[21,22]. Another prospective randomized trial we mentioned above has concluded that the supplement of GnRH agonist associated with higher live birth rates[9]. Due to the advantage of large sample size and close to the clinical practice of the real world, our results will add considerable value to the reassessment of live birth rate for two regimens.

Usually, the 3.75mg GnRH agonist is administered in the mid-luteal phase[23]. In our study, GnRH agonist was administered in the early follicular phase of the previous cycle, and estrogen stimulation was initiated at about 28 days after GnRH agonist administration and continue for 12-14 days. That means the progesterone supplement starting at 40-44 days after GnRH agonist injection and its supression still remains within the time range of the long-acting GnRH agonist effect. We also found that serum LH on the day of progesterone initiation in the agonist group was very low (0.64 (0.35-1.23)). In addition, the negative feedback regulation of the hypothalamo-pituitary axis by high-dose estrogen in the HRT cycle can also effectively inhibit ovulation. Therefore, serum P level on the day of progesterone initiation in

agonist group were less than 1.5ng/ml, which indicates spontaneous ovulation didn't happen[24]. The reason why physicians prefer to use GnRH agonist in 1-4 days of menstruation is that patients can easily arrange their schedules, and avoid pregnancy possibility, but it might happen occasionally if administration in the mid-luteal phase. Both groups received an average of 12.8 days of estrogen stimulation and the serum estrogen level on the day of progesterone initiation were very similar in two groups. This mean that the endometrial proliferation efficiency of the two groups may be similar. The median endometrial thickness on the day of progesterone initiation in both groups reach 9 mm, mostly more than 8 mm, but it was thicker in agonist group. Previous studies shown that endometrial thickness more than 7 mm is sufficient for embryo implantation[25]. The advantage of endometrial thickness in agonist group did not appear to yield clinical outcomes benefit in our study. No difference in clinical pregnancy rates was observed in the other two studies, which also reported a thicker endometrium in the agonist group[11,13].

Stratified analysis showed that the clinical pregnancy rate and the live birth rate decreased with age, and there was no difference in two groups among different age groups. This suggests that the two regimens were equally effective in different age groups. In the first FET cycle, patients in HRT group appeared to have reached a high clinical pregnancy rate and live birth rate. One possible explanation is that patients in the first FET cycle in HRT group had an age advantage, and their median age was less than the average of the overall population (32 VS 33). Physicians usually chose the best quality embryo for transfer in the first FET cycle. Hence, 90.3% of patients in this group had at least one high-quality embryo transfer (52% had two high-quality embryos transfer); only 9.6% of the patients could only transfer the poor quality embryos (shown in Supplement Table 2). In addition, high response patients freezing all embryos because of the risk of OHSS in fresh cycles were likely to administer HRT protocol in their first FET cycle. Those patients were usually in good condition, which might also contribute to the high pregnancy rate in the first FET cycles. The difference in pregnancy rates between the two groups in the first FET cycle may partly account for the significant higher live birth rates in HRT group than HRT with GnRH agonist group. Another possible explanation may be the slightly higher early miscarriage rate in the agonist group. The other three studies also revealed an increase in the miscarriage rate in agonist group, but live birth rate didn't measured in those studies[10,11,13].

The clinical pregnancy rates and live birth rates remained high in both group after three or more FET cycles. The main reason might be most of the embryos frozen were high quality, especially many patients were in younger age when their fresh IVF cycles and oocytes retrieval were carried out. Even in the third or more FET cycles, 85.3% of patients in the HRT group and 86.0% in the HRT with GnRH agonist group transferred at least one high-quality embryo. Besides, if patients had two previous failure cycles of embryo transfer, hysteroscopy or laparoscopy were given for diagnosis and treatment of the unvisualiable abnormal endometrial and uterine cavity pathological changes before proceeding next FET cycle. Therefore, the detection and treatment of endometrial lesions might improve the chances of clinical pregnancy after recurrent implantation failure. In addition, we observed a higher pregnancy rate and live birth rate in HRT with agonist group compared to HRT group in the third or more FET cycle. One reason

was that part of the patients with endometriosis and adenomyosis might be benefit from pituitary suppression by GnRH agonist[26,27]. The other reason was that some patients changed to agonist group after the failure of HRT treatment. Endometrial receptivity might be improvement after administration with GnRH agonist according to previous studies reports[28,29]. It suggested that HRT with GnRH agonist may be optimal for patients with recurrent implantation failure.

The particular strength of our study is its multi-center design and large sample size. Multi-center research can cover a large representative population and the large sample size allowing the great power for statistic analysis. In our study, we evaluated live birth rates and ongoing pregnancy rates that were rarely reported in other studies. The outcome of live birth to a single child are perfectly consistent with clinical practice, the assessment of it will make the evaluation of treatment effect and safety more accurate.We also described the information of the cycles in details, for example, serum hormones level on the day of progesterone initiation and multiple secondary clinical outcomes (ie.,early miscarriage rate, multiple pregnancy rate). But most of the previous studies were lack of such information. Finally, our study is the real-world clinical practice with large sample size, the results are more valuable for reference for routine clinical practice.

However, this study has several limitations. Firstly, its retrospective design limits the interpretation of cause-and-effect. Secondly, a low proportion of the agonist group post a risk of selection bias. Although the two protocol were flexible, it seemed that physicians and patients, even those with poor prognosis, prefer HRT because it takes less time, visit and money. But some patients had received HRT with GnRH agonist because of etiological changes (ie.,adenomyosis and endometriosis) or special schedule needs for patients or doctors (ie. Vacations or work requirement). And some patients changed to agonist group after the failure of HRT treatment. So the proportion of agonist group was low in the overall population. Selection bias is common in retrospective study, but the advantage of large sample size and the application of logistic regression allowed us to adjust a variety of confounding factors for obtaining relatively reliable results. Meanwhile, we screened out standard 2208 patients. The proportion of the agonist group in standard patients was still low, but it was similar with its propotion in overall population (13% vs 18%). That meant even in the standard patients, the physicians' choices of FET protocol were the same as in the whole patients.Besides, there was no differences in clinical outcomes between two groups in standard patients, which can also confirmed our conclusion was reliable. Third, the number of canceled transfers by ovulation was not recorded in our study, which makes it difficult to check the advantage of GnRH agonists in suppressing ovulation during endometrial preparation. Unfortunately, these data were unable tracked due to the retrospective study design. The latest Cochrane review found no evidence of a difference between the two groups in cycle cancellation rate (OR 2.73, 95% CI 0.79 to 9.38, 3 RCTs, n = 636)[15]. The risk of ovulation has been ascribed to a delay in estrogen initiation or to an insufficient estrogen dose. In our study, a large dose of estrogen (6mg daily) supplement was started early days (within 1-4 days)of the menstruation cycle in HRT group, and spontaneous ovulation was more rarely observed in GnRH agonist group because of double supression effects. However, we were unable to confirm this hypothesis due to a lack of data for so few patients.

Conclusions

In conclusion, the present study demonstrates that the artificial endometrium preparation using estrogen and progestin only for FET seems to be as effective as the protocol involving preliminary pituitary suppression with GnRH agonist. Compared with GnRH agonist supplement, the hormonal replacement treatment protocol without GnRH agonist is simpler and quicker to administer. This simplification can reduce pharmacologic treatment and achieve a similar success rate with low cost, thereby improving patient compliance.

Declarations

Acknowledgment

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Author Contributions

WHT conceived the manuscript. MD, YT, FW and LLL were involved in data collection. FW carried out the data analysis. XQZ and GS prepared the tables. FW and LLL drafted and wrote the manuscript. All authors approved the submitted version of the manuscript.

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Data availability

The datasets generated and analysed are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no confict of interest.

Ethical approval: The study was conducted in accordance with the ethical standards for research involving human participants. The Ethical Committee of the The People's Hospital of Guangxi Zhuang Autonomous Region, Women and Children's Hospital of Guangdong Province, and Family Planning Special Hospital of Guangdong Province have approved the research protocol.

Informed consent :We were unable to obtain written informed consent from participants due to the retrospective study design.

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Tables

Table 1: The baseline characteristics of two study groups.

	HRT (n=4143)	HRT with GnRH agonist (n=906)	*p* value
Age (year)	33.0 (29-37)	34.0 (30-38)	<0.001
BMI	21.3 (19.5-23.4)	21.2 (19.5-23.2)	0.58
Basal serum FSH (IU/L) (mIU/ml)	6.7 (5.6-7.9)	6.5 (5.4-7.8)	0.16
Causes of infertility			<0.001
Tubal	62.8% (2603/4143)	62.0% (434/906)	
Diminished ovarian reserve	4.0% (164/4143)	5.5% (50/906)	
Ovulation disorder	2.4% (101/4143)	0.8% (7/906)	
Male factor	18.6% (772/4143)	12.3% (112/906)	
Endometriosis	2.9% (122/4143)	6.0% (54/906)	
AIH failure	9.2% (381/4143)	13.4% (121/906)	
Cycle of embryo transfer			<0.001
First	50.4% (2087/4143)	40.1% (363/906)	
Second	33.4% (1382/4143)	34.7% (314/906)	
Third or more	16.3% (674/4143)	25.3% (229/906)	
Number of high-quality embryo transfer			0.003
One	11.5% (476/4143)	14.7% (133/906)	
Two	40.3% (1670/4143)	42.4% (384/906)	
Three	48.2% (1997/4143)	42.9% (389/906)	
Total estrogen days	12.8 (12-14)	12.8 (12-14.4)	0.142
Total estrogen dose (mg)	98 (83-117)	108 (90-122)	<0.001
Day of progesterone initiation			
Serum estrogen (pg/ml)	239.5 (171.8-399.1)	235.7 (171.2-432.7)	<0.639

Serum progesterone (ng/ml)	0.37 (0.21-0.56)	0.23 (0.09-0.39)	<0.001
Serum luteinizing hormone (mIU/ml)	11.7 (7.1-18.3)	0.64 (0.35-1.23)	<0.001
Endometrial thickness	9.0 (8-10)	10 (8.6-11.1)	<0.001

Table 2: The comparison of clinical outcomes between two study groups.

	HRT (n=4143)	**HRT with GnRH agonist (n=906)**	***p* value**
Implant rate	41.1% (3103/7552)	38.6% (632/1636)	0.067
β-HCG positive rate	61.2% (2534)	61.8% (560/906)	0.72
Clinical pregnancy rate	55.0% (2279/4143)	51.7% (468/906)	0.066
Multiple pregnancy rate	36.9% (841/2279)	35.5% (166/468)	0.84
Early miscarriage rate	16.9% (386/2279)	20.5% (96/468)	0.064
Ongoing pregnancy rate	47.3% (1960/4143)	42.7% (387/906)	0.012
Live birth rate	44.7% (1852/4143)	39.8% (361/906)	0.008

Table 3: Odd ratios for the association between HRT with GnRH agonist and clinical outcomes.

	HRT	**HRT with GnRH agonist OR (95% CI)**		
		Unadjusted	Model 1[a]	Model 2[b]
β-HCG positive	**1**	1.02 (0.88-1.19)	1.14 (0.98-1.33)	0.85 (0.65-1.12)
Clinical pregnancy	**1**	0.87 (0.75-1.01)	0.95 (0.82-1.11)	0.92 (0.70-1.20)
Early miscarriage	**1**	1.29 (0.98-1.68)	1.17 (0.89-1.54)	1.04 (0.66-1.65)
Ongoing pregnancy	**1**	0.83 (0.72-0.96)	0.91 (0.78-1.01)	0.91 (0.69-1.19)
Live birth	**1**	0.83 (0.71-0.96)	0.90 (0.77-1.05)	0.84 (0.64-1.10)

Note

a: Model was adjusted for age.

b: Model was adjusted for age, institution, infertility cause, history of uterine lesions, number of high-quality embryo transplanted, embryo type , transplant times, serum P and serum LH on day of progesterone initiation, and endometrial thickness

Table 4: The comparison of the clinical pregnancy rate and live birth rate between two study groups according to cycle of embryo transfer.

Cycle of embryo transfer	HRT (n=4143)	HRT with GnRH agonist (n=906)	*p* value	OR (95% CI)[a,b]
Clinical Pregnancy Rate				
First	59.2% (1236/2087)	49.3% (179/363)	<0.001	0.69(0.44-1.09)
Second	53.0% (733/1382)	53.5% (168/314)	0.88	1.49(0.94-2.37)
Third or more	46.0% (310/674)	52.8% (121/229)	0.073	0.85(0.49-1.47)
Live Birth Rate				
First	49.5% (1034/2087)	34.7% (126/363)	<0.001	0.49(0.30-0.79)
Second	41.6% (575/1382)	44.9% (141/314)	0.29	1.56(0.98-2.46)
Third or more	36.2% (243/674)	41.0% (94/229)	0.18	0.85(0.49-1.46)

Note

a: Model was adjusted for institution, infertility cause, history of uterine lesions, number of high-quality embryo transplanted, embryo type, serum P and serum LH on day of progesterone initiation, endometrial thickness, and age/ cycle of embryo transfer.

b: HRT group was taken as reference

Table 5: The comparison of clinical outcome between two study groups in standard patients.

	HRT (n=1970)	HRT with GnRH agonist (n=301)	*p* value
β-HCG positive rate	72.4% (1426/1970)	75.1% (226/301)	0.328
Clinical pregnancy rate	66.0% (1301/1970)	63.1% (190/301)	0.321
Early miscarriage rate	8.9% (116/1301)	11.1% (21/190)	0.684
Ongoing pregnancy rate	60.2% (1185/1970)	56.1% (169/301)	0.187
Live birth rate	57.0% (1123/1970)	53.2% (160/301)	0.210

Figures

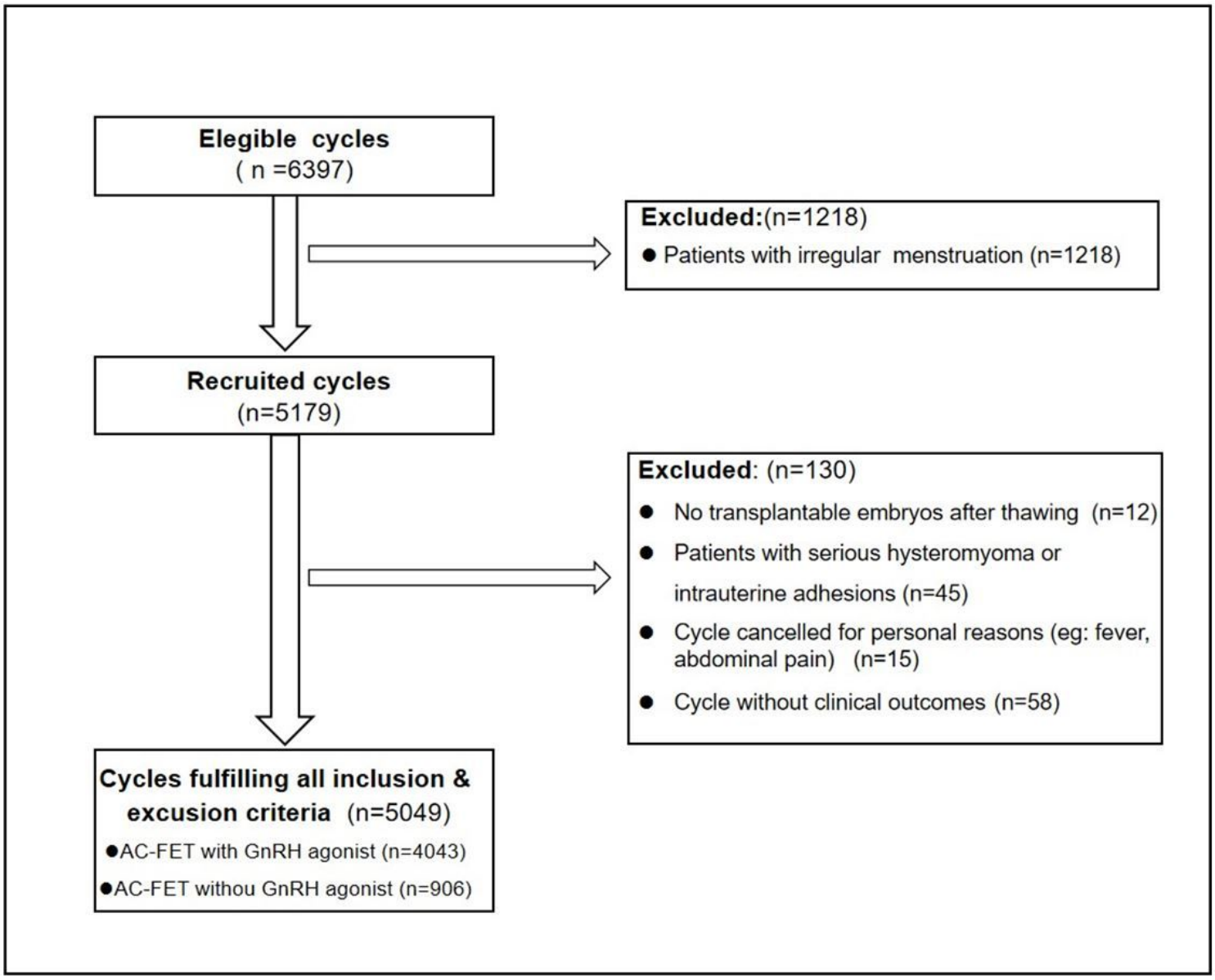

Figure 1

Flowchart of the included cycles.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementTable1and2.docx