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CLINICAL RESEARCH

Clinical Outcomes in One of Four Cycles of Frozen-Thawed Embryo Transfer Cycles were Increased by Administering GnRH-A during the Luteal Phase: Retrospective Analysis

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ABSTRACT

OBJECTIVES

To determine if administration of GnRH-a (triptorelin) as a luteal phase support in the transfer of frozen-thawed embryos (FET) improves pregnancy outcomes.

MATERIALS AND METHODS

we have conducted a Retrospective Cohort study and analyzed 3515 cycles of receiving FET at the Reproductive Center of the Second Affiliated Hospital of Zhengzhou University from February 2018 to December 2021. The Patients were divided into GnRH-a (triptorelin +existing treatment) group and No GnRH-a (existing treatment without Triptorelin) group. There were 1033 and 2485 cases in the study group and control groups. Clinical pregnancy Rate and Live Birth Rate were compared between two groups.

RESULT

We have found higher clinical pregnancy rates (58.0% vs. 48.4%, P = 0.003) and Live Birth Rates (52.7% vs. 45.6%, P = 0.001) for HRT-FET cycles, and found no clinical significance for NC-FET(58.2% vs 52.9%, P=0.364) and (54.4%vs 47.0%,P=0.211), GnRH-a+HRT-FET(53.0% vs 53.0% P=0.176) and(46.2%vs47.3%, P=0.794), and Stimulation-FET (59.3%vs52.9%, P=0.566) and (59.3%vs47.1%, P=0.247) in terms of clinical pregnancy rates and live birth rates between two groups.

There was 47% increment of clinical pregnancy rate in the GnRH-a group and there was 33% increment of live birth rate in the GnRH-a group.

CONCLUSION

During the HRT- FET cycles, administering of Triptorelin 3-4 times in the existing luteal support can improve the clinical pregnancy rate and live birth rate. Administering Triptorelin during the Luteal phase can be a new option for luteal support.

KEYWORDS

Luteal phase support; Hormone replacement treatment; Frozen-thawed embryo transfer; Natural cycle; Assisted reproductive Techniques; Stimulation assisted Cycle; GnRH-A assisted HRT cycle

INTRODUCTION

There are many protocols of endometrium preparation before FET: Natural Cycles, HRT cycles, GnRH-a assisted HRT cycles, Stimulated assisted cycles [1]. Each protocol has its advantages and limitations. FET cycles have gained significance, accounting for up to one third of all children born in the United States, ART technology is used [2].

Currently, there are many medications for luteal support in clinical practice, including progesterone, human chorionic gonadotropin (HCG) and estrogen . Some studies have found that GnRHa is used for luteal support therapy, GnRHa stimulation makes the pituitary gland increases the secretion of Luteinizing hormone (LH) for luteal support. While other researches have shown that There is expression of GnRHa receptors on both sides of placentas, normal endometrium, Myometrium, ovaries and testes (Chegini N Rong H, 1998). It is believed that GnRHa can affect the endometrium local GnRHa receptors exert a direct effect and can improve endometrial receptivity.

At present, GnRHa supplementation in Luteal phase promotes luteal function, embryonic development potential and embryo development, but the mechanism of endometrial receptivity is still unclear.

The study we are presenting is retrospective analysis of FET cycles of patients who are taking treatment at our reproductive center. The aim was to evaluate the effects of triptorelin as luteal phase support for FET Cycles in terms of clinical pregnancy rate and live birth rate and provide a basis for clinical application.

MATERIALS AND METHODS

Research Objective

This study was approved by the ethical committee of the second affiliated hospital of Zhengzhou University and we followed the basic principles of the Declaration of Helsinki. From January 2019 to December 2021, a retrospective study was conducted at the reproductive medicine centre of the second affiliated hospital of Zhengzhou University. We covered all FET-assisted pregnancy protocols. They are Natural Cycle, HRT, GnRH-a +HRT Cycle, and Stimulation Cycle Protocols of FET assisted pregnancy. Retrospective data was obtained. Women's records ranging in age from 20 to 52 were included. The range of their BMIs was 15 to 41.6 kg/m2. AMH ranged from 0 to 59. They experienced infertility for 0.2 to 22 years. They had their own oocytes and embryos. We excluded fresh cycle protocols for assisted pregnancy, oocyte donation cycles, donated embryos, Abnormal uterine environment, such as uterine adhesions and submucosal muscles Tumor, adenomyosis or uterine malformation. Before FET, all patients signed the necessary informed consent forms. We divide the

patients into two groups: one that receives GnRH-a (Triptorelin) during the luteal phase, and the other that does not.

Methods

In our reproductive center, we mostly use the Natural cycle, HRT-FET cycle, GnRHa-HRT cycle, and stimulated cycles to prepare the endometrium for frozen embryo transfer.

In this investigation, we want to assess the effectiveness of GnRH-a as luteal phase support in each of these four FET regimens.

GnRH-a as Luteal phase support in FET protocols is observed to improve clinical outcomes during ART treatments at our reproductive center, but there is no published data to support this. For this reason, we gathered data, conducted a retrospective analysis, and split these protocols into two groups.During the LPS stage in the study group, we employed GnRH-a in addition to other conventional therapies, On the other hand, we didn't administer GnRH-a and instead employed standard LPS methods. The full procedure we followed for this investigation was as follows:

We assessed women's ovulation for candidates with Natural Cycles based on each of their menstrual cycles. Transvaginal ultrasounds were performed on women between the ninth and tenth days of their menstrual cycle. Transvaginal ultrasonography, serum estradiol (E2), and serum luteinizing hormone (LH) were used to track follicular growth. We daily did transvaginal ultrasound examinations until ovulation when the LH level was greater than 20 IU/L. 5000 IU of hCG were administered to initiate oocyte ovulation when the average diameter of the dominant follicle was greater than 17 mm and LH was less than 20 IU/L. On the third day, an embryo transfer was carried out during the cleavage phase. For HRT-FET cycle, to prepare the endometrium, oral estradiol valerate (Progynova; Bayer, Berlin, Germany) was administered daily on days 2-3 of the menstrual cycle at a dose of 6-8 mg. Transvaginal ultrasonography and serum progesterone levels were assessed after 10 to 12 days. 200 mg of vaginally administered micronized progesterone was administered three times daily when the endometrial thickness reached 7 mm or greater and P 1.5 ng/mL, and 20 mg of dydrogesterone was administered orally twice daily for 2-3 days (for cleavage embryos) and 5 days (for blastocyst embryos).

3.75 mg of GnRHa was given into patients as part of the GnRHa-HRT regimen on the second or third day of menstruation during the early Follicular phase.

Regardless of their treatment condition, we required them to return to the hospital after 28 days. The patient's ultrasound results and hormone levels are then used to determine if the patient has reached a state of pituitary downregulation. When levels of estrogen (E2) reached 183.5 pmol/L, follicle stimulating hormone (FSH) reached 5 U/L, luteinizing hormone (LH) reached 5 U/L, endometrial thickness reached 5 mm, and no significant follicle or cyst was seen, the standard criteria for defining down-regulation status was applied. In Stimulated cycle protocol of FET ovulation was induced by medications such as clomiphene citrate, letrozole with or without HMG. The endometrium was prepared by endogenous estrogen and progesterone.

Embryo Thawing Transfer

We defrosted D3 embryos using customary methods for vitrification, and we performed transplanting when more than 50% of the blastomeres survived following thawing.

Luteal Support Method: Frozen Thawed Embryo Transfer

We began providing dydrogesterone on the second day following ovulation, depending on the needs of each patient. Some patients preferred oral drugs, some requested injections, and yet others used vaginal suppository, Up until 14 days following transplantation, the dosage was as follows: 20 mg/d orally and 60–80 mg/day of progesterone by injection, or 200 mg twice daily via vaginal suppository.

For the GnRH-a group, triptorelin acetate (France), 0.1 mg/dose, was injected subcutaneously once on the fourth- or sixth-day following oocyte retrieval (after ovulation) in the basic addition of progesterone and dydrogesterone14 days after transplantation, Triptorelin was terminated while other LPS treatments were continued after being administered four times every three days in addition to the patient's ongoing treatment. In the triptorelin group, after administering 3-4-time triptorelin, administration of existing luteal support medications continues through the 10^{th} to 12^{th} week of pregnancy, while in the non-GnRH-a group, only existing luteal support medications without addition of triptorelin were continued to take during the same period of pregnancy.

On days 35, 55, and 75, the second, third, and fourth pregnancy tests were performed. All luteal support drugs were ceased being administered once ectopic pregnancy was determined to be present or when the pregnancy wasn't found during the test.

Observation Indicators and Follow-Up

After 14-, 35-, 55-, or 75-days following transplantation, patients successfully completed an HCG blood serum pregnancy test. They followed up to the delivery. We looked at their live birth rates and clinical pregnancy rates.

Statistical Analysis

For statistical analysis, we used the SPSS program. Continuous data were reported as means SD. To establish the statistical significance of percentages and odd ratios, we compared the averages using cross-tabs, performed the Pearson Chi-square test, and calculated risk estimates. We defined statistical significance as $P<0.05$ and an odd ratio greater than 1.

RESULTS

We looked at a total of 3518 cycles, 1033 of which were in the GnRH-a group and were given Triptorelin until 10–12 weeks following embryo transfer. Of these 1033 cycles, 587 were noted for clinical pregnancy rate, and 531 for live birth rate. The No GnRH-a group had a total of 2483 cycles; 1277 of those cycles had clinical pregnancies, while 1129 of those cycles had live birth rates reported. They were all treated using the standard practice of luteal phase support after embryo transfers.

Table 1: Comparison of basic indicators between the two groups.

Items	GnRHa group (n=1033)	NoGnRHa group(n=2485)	P value
Age(years)	33.32±5.62	33.46±5.56	0.518
BMI (kg/m2)	23.64±3.59	23.68±23.68	0.76
Duration of infertility (years)	4.49±3.46	4.47±3.51	0.864

AMH	4.36±4.34	4.36±4.27	0.955
AFC	20.61±12.32	21.56±44.53	0.468

Above Table 1 shows basic indicators between the two groups along with p values.

Table 2: Comparison of embryo transfer between two groups.

Items	GnRHa group(n=1033)	NoGnRH group(n=2485)	P value
Endometrial thickness(mm)	9.84±1.92	9.8±2.03	0.598
Total number of transferred embryos	1.73±.444	1.78±0.414	0.001

Table 3: Comparison of pregnancy outcomes between the two groups.

Items	GnRHa group (n=1033)	No GnRH group (n=2485	P value	OR	95% CI	Increment /decrement
CPR(all-FET)	56.8% (n= 587)	51.4% (n=1277)	0.003	1.24	1.08,1.44	24%
LBR(all-FET)	51.4% (n=531)	45.4% (n=1129)	0.001	1.27	1.10,1.47	27%
NC-FET						
CPR	58.2% (n=46)	52.9% (n=1818)	0.364	1.24	0.791.95	24%
LBR	54.4% (n=43)	47.0% (n=1617)	0.211	1.35	0.86,2.11	35%
HRT-FET						
CPR	58.0% (n=391)	48.4% (n=1338)	0.003	1.47	1.24,1.75	47%
LBR	52.7% (n=355)	45.6% (n=1262)	0.001	1.33	1.12,1.57	33%
GnRH-a+HRT-FET						
CPR	53.0% (n=134)	53.0% (n=1730)	0.176	1	0.77,1.29	0.0%
LBR	46.2% (n=117)	47.3% (n=1543)	0.794	0.96	0.74,1.24	-4%
Stimulation-FET						
CPR	59.3% (n=16)	52.9% (n=1848)	0.566	1.30	0.6,2.79	30%
LBR	59.3% (n=16)	47.1% (n=1644)	0.247	1.64	0.76,3.53	64%

Table 3 presents the outcomes after embryo transfer. For HRT-FET cycles, we discovered significant differences in the frequencies of clinical pregnancy (58.0% vs. 48.4%, P = 0.003) and live births (52.7% vs. 45.6%, P = 0.003) between the GnRH-a group and the no GnRH-a group.

Clinical pregnancy rates for the NC-FET, GnRH-a+HRT-FET, and Stimulation-FET cycles were not significantly different between these two groups (58.2% vs 52.9.4%, p=0.364), (53.0% vs 53.0%, p= 0.176), and (59.3% vs 52.9%, p= 0.566), respectively.

The live birth rates for these two groups did not differ significantly for the NC-FET, GnRH-a+HRT-FET, or Stimulation-FET cycles (54.4% vs 47.0, p=0.211), 46.2% vs 47.3%, and 59.3% vs 47.1%, p=0.247), respectively.

In the GnRH-a group, the odds ratio for clinical pregnancy following HRT-FET cycles was 1.47, 95% CI: 1.24, 1.75, and it was statistically significant (P=0.003). The clinical pregnancy rate increased by 47% in the GnRH-a group. In the GnRH-a group, the odds ratio for live birth during HRT-FET cycles was 1.33, 95% CI: 1.12, 1.57, and it was statistically significant (p 0.001). The live birth rate increased by 33% in the GnRH-a group.

DISCUSSION

In four of our FET cycles, HRT-FET appears to be the most effective cycle protocol in terms of clinical pregnancy rates and live birth rates when triptorelin doses are administered during the luteal phase as opposed to the traditional luteal phase support therapy.

The quality of the embryo and the receptivity of the endometrium are the key parameters that affect the success rate of a frozen-thawed embryo transfer (Eidne K, 1987). Natural cycles (NC), hormone replacement cycles (HRT), GnRH-a+HRT cycles, and stimulation cycles can all be used to prepare the endometrium.

For embryo implantation and pregnancy maintenance, the corpus luteum must function normally. COS-related corpus luteum dysfunction can result in a low pregnancy rate, low embryo implantation rate, and a high rate of early miscarriage. As a result, clinical research on the luteal support drugs used in ART treatment is becoming quite popular (Ma Xiaoling, 2019). Although LH secretion in the luteal phase can partially rebound after GnRHa was stopped, progesterone synthesis may not be raised (Beckers NG, 2000). Endometrial biopsy evidence shows that once the endometrial development sheds off, the development of glandular cells slows down following the administration of GnRHa in the middle of the luteal phase. Progesterone levels falling will have an impact on both uterine contraction and endometrial growth. A high frequency of uterine contraction during transplantation can impair embryo placement, prevent implantation, and lower pregnancy rates, according to research using ultrasound to assess the frequency and direction of uterine contraction [5].

In studies involving egg donation and ICSI cycles, Tesarik J, et al. [6] administered 0.1 mg of GnRHa as luteal support on the sixth day after fertilization. These results showed that this treatment significantly increased the implantation rate, pregnancy rate, and birth rate when compared to placebo. This improvement may be explained by the combined effects of GnRHa on the embryo and corpus luteum. Pirard C, et al. [7] employed GnRHa successfully as luteal support in IVF-ET cycles and intrauterine artificial insemination, and they hypothesized that GnRHa would also be useful in ART. GnRHa can boost other pregnancy-related peptides released by the corpus luteum, like relaxin, in addition to just raising progesterone and E2 levels in the blood. LH may directly affect the endometrium, causing it to release cytokines and angiogenic substances that are helpful for embryo implantation. Additionally, it may directly act on the embryo and encourage its development because trophoblastic cells contain GnRHa receptors [6].

The endogenous corpus luteum is at its lowest stage six days following egg retrieval. At this point, GnRHa is used as the corpus luteum's primary support. It binds to the pituitary glands newly produced GnRHa receptor, generating a "flare up" effect that increases the secretion of the ovarian hormones FSH and LH. Increased LH causes granulocytes to secrete more progesterone, which improves ovarian luteal function and makes pregnancy more likely to develop and remain so [8].

Early investigations revealed GnRHa receptor expression in maternal endometrium and human embryonic trophoblast cells. According to Reshef E, et al. [9], functional LH receptors have been identified in human uterine tissue, which raises the possibility that using GnRHa in the middle of the luteal phase will increase the likelihood of clinical pregnancy and facilitate embryo implantation. According to Razieh et al. [10], a single injection of GnRHa in the luteal phase increased the rate of clinical pregnancy and embryo implantation compared to the standard luteal support group.

Human embryos and endometrial stromal cells both have GnRHa receptor mRNA, and giving GnRHa during the middle of the luteal phase can encourage early implantation embryos to secrete hCG. Studies from recent years have suggested using GnRHa as luteal support, however the sample size is relatively small. Future discussions will focus on how the luteal phase support differs from the fresh cycle and how the success rate of frozen embryo transfer cycles has increased due to advances in freeze-thaw technology [10].

Patients who underwent all four FET cycles were chosen for this study. The clinical pregnancy rate and live birth rate of the group with GnRHa (Triptorelin) addition were greater than those of the group without GnRHa addition, with statistically significant differences, according to the findings of HRT-FET cycles. If the administration of GnRHa during the luteal phase increases the risk of fetal birth abnormalities is a topic of interest for several researchers. According to research, using GnRHa in the first trimester of pregnancy does not raise the likelihood of fetal abnormalities (Li Huimin, 2019).

In this study, additional monitoring of the mothers and fetuses had done to see if the use of GnRHa during the luteal phase raises the risk of fetal birth abnormalities.

In this study, additional monitoring of the mothers and fetuses will be done to see if the use of GnRHa during the luteal phase raises the risk of fetal birth abnormalities.

CONCLUSION

Supplementing with GnRHa during the luteal phase can increase the clinical pregnancy rate and live birth rate, and it may also open up new possibilities for luteal support. In our center, this study is, however, only on a small scale. To further compare the variations in the use of GnRHa in various freeze-thaw schemes, the selection of treatment population, the use dose of GnRHa, the time and frequency of administration, and have obtained a unified standard for the effective luteal support of GnRHa, it is suggested that we conduct RCT on a large sample of the center. At the same time, we must consider how GnRHa use affects perinatal children.

DATA AVAILABILITY STATEMENT (DAS)

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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There is no funding for this study as it is retrospective cohort study, it means patients have regular treatments at our reproductive medicine center and we stored their regimens and treatment course and information, and we have compiled this data for two years and conducted analysis, it is different than trial where patients are recruited for study.

AUTHOR CONTRIBUTION

M.A. participated in the study design and analysis and in drafting the article. J.Z. and Y.L. participated in the acquisition and analysis of data. L.T. gave critical advice in the study design. M.A. were corresponding author and they participated in the study conception and design. All of us approve the version to be published.

CONFLICT OF INTEREST

we have no commercial interest with anyone, it is pure study for the benefits of patients and physicians.

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