

Original Research

Gonadotropin-Releasing Hormone Agonist Combined with Hormone Replacement Therapy Protocol Improves the Live Birth Rate in Frozen-Thawed Embryo Transfer Cycles for Patients without Endometriosis

Yu Wang¹, Huixia Xie¹, Ping Shen², Qun Lv^{1,*} ¹Department of Reproductive Medicine Center, Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China, 610072 Chengdu, Sichuan, China²Department of Obstetrics and Gynecology, Shuangliu District First People's Hospital, 610200 Chengdu, Sichuan, China*Correspondence: lqwmzy@163.com (Qun Lv)

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Abstract

Background: Whether gonadotropin-releasing hormone agonist (GnRH-a) pituitary suppression improves clinical outcomes in non-endometriosis patients undergoing frozen embryo transfer remains controversial. The objective of this study is to investigate whether GnRHa combined with hormone replacement treatment (HRT) compared to HRT alone can improve the clinical outcomes of frozen-thawed embryo transfer in patients without endometriosis. **Methods:** This is a retrospective cohort study. This study involved 2178 frozen-thawed embryo transfer (FET) cycles of non-endometriosis patients between January 2018 and December 2019, of these cycles, 1535 were GnRHa-HRT combined cycles and 643 were HRT alone cycles. The primary outcomes were the clinical pregnancy and live birth rates. SPSS software (version 23.0, IBM Corp., Chicago, IL, USA) was used for data analysis. **Results:** Single-factor analysis showed that the live birth and implantation rates -were higher in the GnRHa-HRT group than those in the HRT group ($p < 0.05$). The mid-to-late-term miscarriage rate in the GnRHa-HRT group was lower than that in the HRT group ($p < 0.05$). The rates of human chorionic gonadotropin (HCG) positivity, clinical pregnancy, early abortion, multiple pregnancy, and preterm delivery between the two groups were comparable. Multivariate logistic regression analysis showed that rate of the live birth in the GnRHa-HRT group was higher than in the HRT group ($p = 0.009$), and there was no significant difference in the clinical pregnancy rate between the two groups ($p = 0.103$). **Conclusions:** This large-scale retrospective study revealed that non-endometriosis women in FET cycles may benefit from the GnRHa downregulation due to increasing the live birth rate.

Keywords: gonadotropin-releasing hormone; frozen-thawed embryo transfer; live birth pregnancy rate; hormone replacement therapy

1. Introduction

Infertility is estimated to affect approximately one in six people around the world at some point in their lives [1]. *In vitro* fertilization-embryo transfer (IVF-ET) and related techniques are among the most effective treatments for infertility. Patients undergoing fresh cycles may exhibit endometrial abnormalities, high progesterone, pelvic infection after oocyte retrieval, and other complications, which can result in poor clinical outcomes [2]. On the other hand, frozen-thawed embryo transfer (FET) is performed to provide a better implantation environment and to avoid wasting embryos. In addition, whole embryo freezing plays a crucial role in reducing the risk of ovarian hyperstimulation syndrome, ensuring the safety of IVF [3]. Therefore, FET has become an essential treatment option for assisted reproductive technology (ART).

The success of FET is determined by three key factors: embryo quality, endometrial receptivity, and embryo-endometrium synchronization [4]. With improvements in the technique of frozen-thawed embryos, damage to the em-

bryos caused by freezing and thawing has been reduced. Therefore, endometrium receptivity has become a hot topic in the study of frozen embryos.

The endometrial preparation protocols for the FET cycles may affect endometrial receptivity. Commonly used methods to prepare the endometrium include nature cycle (NC), hormone replacement treatment (HRT), ovarian stimulation, and hormone replacement treatment combined with GnRH pretreatment (GnRHa-HRT). HRT has become the most popular method of endometrial preparation in reproductive centers due to its low time restriction and cycle cancellation rate. Gonadotropin-releasing hormone agonist (GnRH-a) exhibits therapeutic effects on endometriosis by inhibiting the hypothalamic-pituitary-ovarian axis so it is considered to improve the clinical outcomes of frozen embryo transfer in patients with endometriosis theoretically, which has been proved in some clinical studies [5,6]. However, the role that GnRH-a plays in patients undergoing FET without endometriosis, is still controversial.



Some studies on FET revealed that GnRHa-HRT promoted endometrial receptivity. A recent retrospective cohort study [7] indicated that the GnRHa pretreatment can increase live birth, ongoing pregnancy, and clinical pregnancy rates in patients with advanced age and recurrent implantation failure. A single-blind randomized controlled trial (RCT) by Aghahoseini *M et al.* [8] in patients with hyperandrogenic polycystic ovary syndrome (PCOS) found a higher rate of ongoing pregnancy and a lower rate of miscarriage in the GnRHa downgrading group in the frozen embryo cycles.

But an RCT study [9] that included 188 patients with PCOS showed that there were no significant differences in pregnancy outcomes, incidence of obstetric complications and neonatal malformations between the GnRHa-HRT and HRT groups. Another RCT study [10] included 236 frozen-thawed embryo transfer cycles, randomized to the modified natural group and the GnRHa-HRT group, and all the patients transferred only one euploid blastocyst. The results of the study showed that the clinical pregnancy, miscarriage, and live birth rates of the two groups were comparable.

The number of participants in the above RCT studies was small. In this study, 2178 FET cycles of GnRHa-HRT or HRT were retrospectively analyzed to investigate the clinical outcome of patients treated with two different endometrial preparation protocols. All patients were diagnosed as non-endometriotic.

2. Materials and Methods

2.1 Study Population

This is a retrospective study included 2178 FET cycles performed between January 2018 and December 2019 at the Center of Assisted Reproductive Medicine Center of Sichuan Provincial People's Hospital. The inclusion criteria were as follows: (1) patients with endometrial preparation using GnRHa-HRT or HRT, (2) patients under 40 years of age. The exclusion criteria were as follows: (1) patients with endometriosis or/and adenomyosis, (2) patients with a body mass index (BMI) over 30 kilograms per square meter, (3) patients with hydrosalpinx on one or both sides, (4) patients with intrauterine adhesion, (5) patients with congenital malformations of the uterus, (6) patients with uterine fibroids, (7) chromosome abnormalities of either one of the couples, (8) patients who received oocytes. Of these 2178 FET cycles, 1535 were GnRHa-HRT cycles and 643 were HRT cycles. The decision to implement GnRHa-HRT or HRT was based on the physician's approval of the protocol, the patient's time and affordability, and previous embryo transfer history. Informed consent was obtained from all subjects prior to their participation in the study, in accordance with the Declaration of Helsinki. The research protocol was approved by the Reproductive Ethics Committee of Sichuan Provincial People's Hospital (NO. 202209).

2.2 Endometrial Preparation Protocols

2.2.1 HRT Group

Daily oral administration of estradiol valerate was 3 mg (Progynova, Bayer Schering Pharma, Berlin, Germany) twice a day starting from the 2nd or 3rd day of menstruation. Additional doses, starting from 2 mg/d were given if the endometrial thickness was <8 mm, 7 days after initial administration. The endometrial thickness was then checked every 3–5 days to determine the doses of Progynova. The maximal dose was 10 mg/d. Luteal support was given when the endometrial thickness is ≥ 8 mm and plasma E2 ≥ 450 pmol/L.

2.2.2 GnRHa-HRT Group

3.75 mg triptorelin acetate (Diphereline, Ipsen Pharma Biotech, Paris, France) was administered by injection at a dose of 3.75 mg on the 2nd–5th day of menstruation. Progynova was given in the HRT cycles after 28 days.

2.3 Luteal Support

In both the two groups, luteal support was started 4 days before the transfer of the split embryo and 5 days before the transfer of the blastocyst. Luteal support was achieved by daily injection of 60 mg progesterone (Zhejiang Xianju Pharmaceutical Co Ltd., Taizhou, Zhejiang, China). Alternatively, a 90 mg daily progesterone sustained-release vaginal gel (Crinone, Fleet Laboratories Ltd., Watford, United Kingdom) vaginally.

2.4 Embryos Cryopreservation and Throwing

All embryos were cryopreserved using the vitrification method.

In the afternoon 1 day prior to transfers, the D3 embryos were thawed. On the morning of the embryo transfer day, the D5/D6 blastocysts were thawed. The survival of more than half of blastomeres was defined as embryonic survival; While the absence of blastomere damage was defined as whole embryonic survival.

2.5 Pregnancy Outcomes

Biochemical pregnancy was diagnosed by serum β -HCG ≥ 10 IU/L after 12 days of transfer.

Clinical pregnancy was defined as observation of a gestational sac via ultrasound after 30 days of transfer. The embryo implantation rate was the ratio of the number of gestational sacs to the number of embryos transferred.

2.6 Statistical Analysis

The SPSS software (version 23.0, IBM Corp., Chicago, IL, USA) was used for data analysis. Continuous variables were described as the mean \pm standard deviation (mean \pm SD), and the two groups were compared using an independent samples *t*-test. Categorical variables expressed as percentages (%), were compared using a chi-square test. A difference of $p < 0.05$ was regarded as a

Table 1. Baseline characteristics of patients in the two groups.

Characteristic	GnRHa-HRT (n = 1535)	HRT (n = 643)	χ^2/t value	<i>p</i> value
Female age (y)	30.29 ± 3.81	29.75 ± 3.77	-3.052	0.002
BMI (kg/m ²)	22.06 ± 2.30	22.08 ± 2.27	0.143	0.886
Infertility type (n,%)			0.947	0.330
Primary infertility	800 (52.1)	320 (49.8)		
Secondary infertility	735 (47.9)	323 (50.2)		
The endometrial thickness on the day of transfer (mm)	10.16 ± 1.63	9.48 ± 1.33	-10.164	<0.001
The mean number of embryos transferred	1.76 ± 0.45	1.73 ± 0.46	-1.142	0.254
Blastocyst transfer rate	73.50%	73.30%	0.013	0.910

Values represent means ± SD or the number (percentage) of patients.

GnRHa, gonadotrophin-releasing hormone agonist; HRT, hormone replacement treatment; BMI, bodymass index.

Table 2. Pregnancy outcomes in the two endometrial preparation groups.

Pregnancy outcomes	GnRHa-HRT (n = 1535)	HRT (n = 643)	<i>p</i> value
HCG positivity rate (%)	70.2 (1078/1535)	66.1 (425/643)	0.057
Clinical pregnancy rate (%)	60.5 (928/1535)	56.6 (364/643)	0.096
Embryos implantation rate (%)	44.1 (1191/1115)	38.6 (430/2669)	0.002
First-trimester miscarriage rate (%)	11.0 (102/928)	12.9 (47/364)	0.331
Mid-to-late-term miscarriage rate (%)	1.7 (16/928)	3.6 (364)	0.044

Values represent percentages (no./no.) of the patients.

HCG, human chorionic gonadotropin; GnRHa, gonadotrophin-releasing hormone agonist; HRT, hormone replacement treatment.

statistically significant difference. The relationship of each parameter to clinical pregnancy and live birth rates was analyzed using multivariable logistic regression. The included covariates were endometrial preparation protocol, age, type of infertility, BMI, endometrial thickness, and the number of embryos transferred.

3. Results

3.1 Baseline Characteristics of Patients

Table 1 provides a description of the patient's baseline characteristics. The BMI, mean number of embryos transferred, the ratio of primary infertility to secondary infertility, and the blastocyst transfer rate between the two groups were not significant different. There was a significant difference ($p < 0.05$) in the average age between the two groups, and the GnRHa-HRT group was higher than the HRT group. On the day of embryo transfer, the endometrial thickness was significantly thicker ($p < 0.05$).

3.2 The Pregnancy Outcomes

Table 2 showed the pregnancy outcomes. The HCG positivity, clinical pregnancy, and first-trimester abortion rates were not significant different between the two groups. However, there was a statistically significant difference ($p < 0.05$) in the implantation rate which was higher in the GnRHa-HRT group (44.1%) compared to the HRT group (38.6%). Additionally, the rate of mid-to-late-term miscarriage was lower in the GnRHa-HRT group (1.7%) compared to the HRT group (3.6%), which was also statistically significant ($p < 0.05$).

3.3 The Obstetric Outcomes

Table 3 showed the patients' obstetrical outcomes. There was a higher rate of live birth rate in the GnRHa-HRT group (52.3%) compared to the HRT group (46.3%), showing statistically significant differences ($p < 0.05$). In the GnRHa-HRT group, 680 of 928 were singleton pregnancies and 248 of 928 were twin or triple pregnancies. In the HRT group, 275 of 364 were singleton pregnancies and 89 of 364 were twin or triple pregnancies. The rate of multiple pregnancies was comparable between the two groups. The rate of preterm delivery rate was not statistically significant different between the two groups (28.0% vs. 24.2%, $p = 0.2$).

3.4 Multifactor Logistic Regression Analysis of Factors Associated with FET Outcomes

Multivariate logistic regression analysis was conducted with clinical pregnancy and live birth rates as dependent variables and endometrial preparation protocol, average age, endometrial thickness on embryo transfer day, number of embryos transferred, BMI and infertility type as independent variables. Table 4 showed the results. Clinical pregnancy and live birth rates were significantly affected by mean age and number of embryos transferred. Endometrial thickness, BMI, and infertility type did not influence live birth and clinical pregnancy rates. Clinical pregnancy rate did not differ significantly between the two methods of endometrial preparation, but the GnRHa-HRT group had a higher live birth rate than the HRT group.

Table 3. The obstetric outcomes of the two groups.

Obstetric outcomes	GnRHa-HRT (n = 1535)	HRT (n = 643)	p value
Multiple pregnancy rate (%)	26.7 (248/928)	24.5 (89/364)	0.402
Live birth rate (%)	52.3 (803/1535)	46.3 (298/643)	0.011
Preterm delivery rate (%)	28.0 (225/803)	24.2 (72/298)	0.2

Values represent percentages (no./no.) of the patients.

GnRHa, gonadotrophin-releasing hormone agonist; HRT, hormone replacement treatment.

Table 4. An analysis of factors related to clinical pregnancy and live birth rates using multifactor logistic regression analysis.

Independent variables	Clinical pregnancy rate		Live birth rates	
	OR value (95% CI)	p value	OR value (95% CI)	p value
Endometrial preparation protocols				
HRT	Ref.		Ref.	
GnRHa-HRT	1.174 (0.968–1.424)	0.103	1.289 (1.065–1.560)	0.009*
Female age (y)	0.953 (0.931–0.976)	<0.001*	0.949 (0.928–0.972)	<0.001*
The endometrial thickness on the day of transfer	1.021 (0.965–1.081)	0.467	1.005 (0.950–1.063)	0.868
BMI	1.011 (0.973–1.050)	0.587	1.003 (0.966–1.042)	0.865
Infertility type				
Primary infertility	Ref.		Ref.	
Secondary infertility	1.166 (0.976–1.393)	0.091	1.106 (0.928–1.318)	0.258
Number of embryos transferred	1.491 (1.231–1.805)	<0.001*	1.503 (1.241–1.820)	<0.001*

* $p < 0.05$ for logistic regression.

CI, confidence interval; OR odds ratio; BMI, body mass index; GnRHa, gonadotrophin-releasing hormone agonist; HRT, hormone replacement treatment.

4. Discussion

FET has become an essential aspect of ART. A successful frozen embryo transfer is determined by three key factors: embryo quality, endometrial receptivity, and embryo-endometrium synchronization [4]. As the methods of endometrial preparation are crucial to endometrial receptivity, it is a fundamental step of the FET.

The nature cycle (NC), the hormone replacement treatment (HRT), the ovarian stimulation, and hormone replacement treatment with the gonadotrophin-releasing hormone agonist pretreatment (GnRHa-HRT) are commonly used to prepare the endometrium [11]. The nature cycle (NC) is the most physiological method and is liable to be performed with fewer costs, but the cycle cancellation rate is high [12]. Women suffering from ovulatory dysfunction with insensitivity to orally administered estrogens or contraindication of estrogens might benefit from ovarian stimulation. The hormone replacement treatment is the most popular FET method of preparing the endometrium due to its extensive clinical application and time flexibility of transfer scheduling [13]. The hormone replacement treatment is categorized into GnRHa-HRT (Pituitary down-regulation by injecting GnRHa) and HRT. The GnRHa protocol is known to improve the clinical outcome in patients with endometriosis due to the therapeutic effects of GnRHa on endometriosis, but the most effective endometrial preparation protocol remains controversial.

A Cochrane review [14] findings showed that the GnRHa-HRT protocol resulted in a higher live birth rate than the HRT protocol, but neither miscarriage nor ongoing pregnancy. A study [15] showed that GnRHa downregulation is associated with higher rates of clinical pregnancy and live birth rates in FETs with male-factor infertility. Another RCT [16] showed that the GnRHa-HRT group and the HRT group did not differ in clinical pregnancy rates, implantation rates, early pregnancy loss rates, or live birth rates.

However, these two RCT studies had small sample sizes. In this study, it was revealed that there was a significant increase in live birth rate with the GnRHa-HRT protocol, but the clinical pregnancy rate was comparable between the two groups. According to previous studies, the possible reasons for the increased pregnancy outcome of FET when using GnRHa-HRT are as follows: Hormone replacement therapies may still result in the development of follicles that produce endogenous luteinising hormone (LH), which could lead to an earlier implantation window. However, the administration of GnRHa prevented follicular growth by suppressing the pituitary [17]. It was found that GnRHa downregulation significantly increased mRNA and protein expression of HOXA10, MEIS1 and LIF in endometrium [18]. HOXA10, MEIS1 and LIF are marks of endometrial receptivity. There has been research showing that GnRHa increases cytokinesis and integrin expression in endometrium. Neukomm *et al.* [19] revealed that the increased number of cytokinesis on endometrial cells and up-regulated integrins of endometrium were proved to

enhance endometrial receptivity. Research [20] showed that GnRH-a could directly bind to its receptors on the endometrium to regulate decidual endometrial stromal cell motility, thereby improving embryo implantation. Studies showed that endometrial cells expressed higher levels of adhesion molecules when GnRHa bound to its receptors on the endometrium, to inhibit the production of embryotoxic autoantibodies, and increase the implantation rate [21,22]. The results of a recent retrospective study [23] showed that GnRHa pretreatment can improve the clinical pregnancy and live birth rates in FET cycles. In addition, the study examined the mRNA expression of cytokines associated with endometrial receptivity in the endometrium of patients whose transfer was canceled on the day of FET. The findings of the study suggest that pretreatment with GnRHa significantly increases the expression of IL-6, IL-11, LIF and integrin $\alpha v \beta 3$ mRNAs in the endometrium. Compared to the group without GnRHa pretreatment, the expression of these mRNAs and markers was significantly higher in the groups with GnRHa pretreatment. These results suggest the potential utility of GnRHa pretreatment in the regulation of endometrial receptivity.

5. Conclusions

Although several RCTs have investigated whether GnRHa downregulation affects treatment outcomes in FET cycles for patients without endometriosis, results have varied, even for patients with the same infertility etiology, creating confusion in the clinical setting for clinicians. Additionally, these previous RCTs have suffered from small sample sizes. Therefore, it is necessary to conduct more research to clarify the confusion surrounding how GnRHa downregulation may affect the outcomes of FET. The greatest strength of our study is its large sample size. Using a larger sample size is more accurate because it captures a broader range of patients and can better represent the entire patient population. Our study revealed that in the FET cycles, a significantly increase in live birth rate was observed among GnRHa-HRT among GnRHa-HRT patients without endometriosis when compared with HRT patients. However, these findings need to be confirmed by large-scale prospective randomized controlled trials in the future. It is also necessary to analyse patients in groups according to different infertility etiologies.

Availability of Data and Materials

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

Author Contributions

QL: design research and revision of paper. YW: collate original data and write paper, and was a major contributor in writing the manuscript. HXX: collate original data. PS: application of statistical to analyze study data. All

authors contributed to editorial changes in the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Informed consent was obtained from all subjects prior to their participation in the study, in accordance with the Declaration of Helsinki. The research protocol was approved by the Reproductive Ethics Committee of Sichuan Provincial People's Hospital (NO. 202209).

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Conflict of Interest

The authors declare no conflict of interest.

References

- [1] The World Health Organization. Infertility prevalence estimates (1990-2021). 2023. Available at: <https://www.who.int/health-topics/infertility> (Accessed: 3 April 2023).
- [2] Zhang W, Xiao X, Zhang J, Wang W, Wu J, Peng L, *et al.* Clinical outcomes of frozen embryo versus fresh embryo transfer following in vitro fertilization: a meta-analysis of randomized controlled trials. *Archives of Gynecology and Obstetrics*. 2018; 298: 259–272.
- [3] Massart P, Sermondade N, Dupont C, Sifer C, Cedrin-Dumerin I, Hugues JN. Elective cryopreservation of all embryos in women at risk of ovarian hyperstimulation syndrome: prevention and efficiency. *Gynecologie, Obstetrique & Fertilité*. 2013; 41: 365–371.
- [4] Edwards RG. Implantation, interception and contraception. *Human Reproduction*. 1994; 9: 985–995.
- [5] Niu Z, Xia L, Chen Q, Feng Y. Selection of endometrial preparation plan for adenomyosis freeze-thaw embryo transfer cycle. *Journal of Reproductive Medicine*. 2011; 20: 197–200.
- [6] Niu Z, Chen Q, Sun Y, Feng Y. Long-term pituitary downregulation before frozen embryo transfer could improve pregnancy outcomes in women with adenomyosis. *Gynecological Endocrinology*. 2013; 29: 1026–1030.
- [7] Pan D, Yang J, Zhang N, Wang L, Li N, Shi J, *et al.* Gonadotropin-releasing hormone agonist downregulation combined with hormone replacement therapy improves the reproductive outcome in frozen-thawed embryo transfer cycles for patients of advanced reproductive age with idiopathic recurrent implantation failure. *Reproductive Biology and Endocrinology*. 2022; 20: 26.
- [8] Aghahoseini M, Alyasin A, Rashidi S, Samaei-Nouroozi A, Saeidi H, Shabani-Nashtaei M. The efficacy of gonadotropin-

releasing hormone (GnRH) agonist before frozen embryo transfer in improving pregnancy outcome and decreasing miscarriage rate in hyperandrogenic polycystic ovary syndrome women: a randomized clinical trial. *Minerva Ginecologica*. 2020; 72: 212–218.

- [9] Salemi S, Yahyaei A, Vesali S, Ghaffari F. Endometrial preparation for vitrified-warmed embryo transfer with or without GnRH-agonist pre-treatment in patients with polycystic ovary syndrome: a randomized controlled trial. *Reproductive Biomedicine Online*. 2021; 43: 446–452.
- [10] Greco E, Litwicka K, Arrivi C, Varricchio MT, Caraglia A, Greco A, *et al*. The endometrial preparation for frozen-thawed euploid blastocyst transfer: a prospective randomized trial comparing clinical results from natural modified cycle and exogenous hormone stimulation with GnRH agonist. *Journal of Assisted Reproduction and Genetics*. 2016; 33: 873–884.
- [11] Mumusoglu S, Polat M, Ozbek IY, Bozdogan G, Papanikolaou EG, Esteves SC, *et al*. Preparation of the Endometrium for Frozen Embryo Transfer: A Systematic Review. *Frontiers in Endocrinology*. 2021; 12: 688237.
- [12] Mackens S, Santos-Ribeiro S, van de Vijver A, Racca A, Van Landuyt L, Tournaye H, *et al*. Frozen embryo transfer: a review on the optimal endometrial preparation and timing. *Human Reproduction*. 2017; 32: 2234–2242.
- [13] Dunselman GAJ, Vermeulen N, Becker C, Calhaz-Jorge C, D’Hooghe T, De Bie B, *et al*. ESHRE guideline: management of women with endometriosis. *Human Reproduction*. 2014; 29: 400–412.
- [14] Ghobara T, Gelbaya TA, Ayeleke RO. Cycle regimens for frozen-thawed embryo transfer. *The Cochrane Database of Systematic Reviews*. 2017; 7: CD003414.
- [15] Yu J, Chen P, Luo Y, Lv M, Lou L, Xiao Q, *et al*. GnRH-agonist pretreatment in hormone replacement therapy improves pregnancy outcomes in women with male-factor infertility. *Frontiers in Endocrinology*. 2022; 13: 1014558.
- [16] Xu J, Li SZ, Yin MN, Liang PL, Li P, Sun L. Endometrial Preparation for Frozen-Thawed Embryo Transfer With or Without Pretreatment With GnRH Agonist: A Randomized Controlled Trial at Two Centers. *Frontiers in Endocrinology*. 2021; 12: 722253.
- [17] Groenewoud ER, Cantineau AE, Kollen BJ, Macklon NS, Cohen BJ. What is the optimal means of preparing the endometrium in frozen-thawed embryo transfer cycles? A systematic review and meta-analysis [published correction appears in *Human Reproduction Update*. 2017; 23: 255–261]. *Human Reproduction Update*. 2013; 19: 458–470.
- [18] Xu B, Geerts D, Hu S, Yue J, Li Z, Zhu G, *et al*. The depot GnRH agonist protocol improves the live birth rate per fresh embryo transfer cycle, but not the cumulative live birth rate in normal responders: a randomized controlled trial and molecular mechanism study. *Human Reproduction*. 2020; 35: 1306–1318.
- [19] Neukomm C, Mueller MD. New insights into the pathophysiology of endometriosis. *Gynakologisch-Geburtshilfliche Rundschau*. 2007; 47: 113–117. (In German)
- [20] Wu HM, Huang HY, Lee CL, Soong YK, Leung PCK, Wang HS. Gonadotropin-releasing hormone type II (GnRH-II) agonist regulates the motility of human decidua endometrial stromal cells: possible effect on embryo implantation and pregnancy. *Biology of Reproduction*. 2015; 92: 98.
- [21] Khan KN, Kitajima M, Hiraki K, Fujishita A, Nakashima M, Ishimaru T, *et al*. Cell proliferation effect of GnRH agonist on pathological lesions of women with endometriosis, adenomyosis and uterine myoma. *Human Reproduction*. 2010; 25: 2878–2890.
- [22] Wu XY, Li ZL, Lin H, Xiao WF, Xiao JF, Liu RJ. Outcome analysis of three kinds of Endometrial preparation programs for frozen-thawed embryo transfer in patients with multiple embryo transfer failures. *Chinese Journal of Family Planning*. 2014; 22: 248–251.
- [23] Li L, Liu L, Kou Z, Huo M, An J, Zhang X. GnRH agonist treatment regulates IL-6 and IL-11 expression in endometrial stromal cells for patients with HRT regimen in frozen embryo transfer cycles. *Reproductive Biology*. 2022; 22: 100608.