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ORIGINAL RESEARCH

Gonadotropin Releasing Hormone Agonists Combined with Hormone Replacement Therapy Significantly Improves Reproductive Outcomes for Patients with Thin Endometrium and Intramural Fibroids in Frozen Embryo Transfer Cycles

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Background: Both intramural myomas and thin endometrium exert a detrimental influence on the outcomes of assisted reproductive technology (ART). The downregulation of gonadotropin releasing hormone agonists (GnRH-a) is regarded as an effective approach to reducing the size of intramural fibroids and enhancing endometrial receptivity. Consequently, we conducted this study to assess whether the GnRH-a combined with hormone replacement therapy (GnRH-a-HRT) can improve reproductive outcomes in frozen embryo transfer cycles for patients with a thin endometrium (≤7 mm) and intramural fibroids.

Methods: This retrospective cohort study encompassed 360 patients who underwent frozen embryo transfer following in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles. Patients were stratified into three groups based on the endometrial preparation protocol: the natural cycle (NC) group (n=96), the hormone replacement therapy (HRT) group (n=180), and the GnRH-a-HRT group (n=84). The live birth rate (LBR) was designated as the primary outcome, while clinical pregnancy rate (CPR), miscarriage rate, and ectopic pregnancy rate were classified as secondary outcomes.

Results: The LBR and CPR in the GnRH-a-HRT group were significantly higher than those in both the HRT group and the NC group (both $P < 0.0001$). A logistic regression model indicated that the LBR was significantly higher in the GnRH-a-HRT group compared to both the HRT group (odds ratio, 0.269; 95% confidence interval, 0.114–0.637; $P = 0.003$) and the NC group (odds ratio, 0.524; 95% confidence interval, 0.457–0.956; $P = 0.023$). Subgroup analyses based on the number and dimension of fibroids demonstrate the positive efficacy of the GnRH-a–HRT regimen.

Conclusion: Compared to NC and HRT protocol, improved reproductive outcomes were observed in the GnRH-a–HRT group. These findings provide valuable insights for exploration of the underlying mechanisms by which the GnRH-a-HRT protocol enhances reproductive outcomes in patients of thin endometrium with intramural fibroids.

Keywords: frozen embryo transfer, live birth rate, clinical pregnancy rate, thin endometrium, intramural fibroid

Introduction

Since the advent of assisted reproductive technology (ART), clinicians and researchers have sought to enhance their procedures with a singular objective: to enhance the live birth rate (LBR). Endometrial thickness (EMT) is a pivotal factor affecting endometrial receptivity, which is essential for optimizing the LBR.[1] A thin endometrium is typically defined as having an EMT of less than 7 mm, with its prevalence ranging from 2.4% to 8.5%.[2,3] Within the realm of ART, frozen embryo transfer (FET) is regarded as a pivotal strategy for patients with thin endometrium.[4,5] The methodologies for preparing frozen embryo transfer (FET) can be primarily classified into three categories: natural cycle (NC), hormone

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replacement cycle (HRT), and GnRH agonist (GnRH-a) downregulation in combination with HRT (GnRH-a -HRT).[6,7] GnRH-a can downregulate pituitary function, and numerous studies have demonstrated that GnRH-a suppression prior to HRT significantly enhances the probability of achieving pregnancy.[8,9]

Concerning intramural fibroids, researchers contend that intramural myomas exert a secondary influence on the outcomes of ART.[10,11] Recently, GnRH-a has been increasingly employed in patients with intramural fibroids to diminish the magnitude of the fibroids and to mitigate their recurrence following surgical intervention.[12,13] Furthermore, given the positive impact of GnRH-a on endometrial receptivity and their therapeutic effects on intramural fibroids, we hypothesize that a GnRH-a-HRT protocol could enhance reproductive outcomes in individuals with thin endometrium and intramural fibroids undergoing FET. If validated, this individualized medical treatment plan would establish a foundation for clinical interventions aimed at maximizing patient benefits. However, there remains a paucity of reliable evidence to substantiate this hypothesis.

In the present study, we performed a retrospective cohort analysis to evaluate three endometrial preparation protocols and to determine whether a more suitable regimen exists for patients with thin endometrium and uterine fibroids during FET.

Materials and Methods

Study Design and Participants

This study retrospectively included 360 FET cycles conducted at the People's Hospital of Zhengzhou University between January 2017 and December 2023. The following inclusion criteria were applied: (1) individuals diagnosed with at least one intramural myoma via ultrasound; and (2) EMT $\leq$ 7 mm on the day of transfer.

The exclusion criteria were as follows: (1) cycles canceled for various reasons; (2) untreated individuals with hydrosalpinx, submucous fibroids, uterine adhesions, endometrial polyps, and uterine malformations that may affect pregnancy success rates; (3) Women with fibroids larger than 5 cm in diameter were excluded; (4) history of myomectomy; (5) individuals who encountered FET following preimplantation genetic testing (PGT) or those presenting with chromosomal disorders; (6) individuals diagnosed with endometriosis or adenomyosis; (7) individuals experiencing recurrent spontaneous abortion (RSA) and recurrent implantation failure (RIF); (8) individuals with comorbidities contraindicating pregnancy, such as severe cardiovascular history, liver disease, kidney disease, uncontrolled immune disorders, and gynecological malignancies; and (9) patients diagnosed as polycystic ovary syndrome (PCOS).

RSA is characterized by the occurrence of two or more pregnancy terminations before the 20th week of gestation.[14] RIF is characterized by the inability to attain clinical pregnancy following the transfer of a minimum of three high-quality embryos across three frozen or fresh cycles.[15] In this context, a high-quality embryo is defined as a day 3 embryo with at least 8 cells, exhibiting symmetry and less than 10% fragmentation,[16] or a blastocyst graded $\geq$3BB.[17]

Women did not sign an informed consent since the study was retrospective. However, all women who were referred to our unit provided an informed consent for their data to be used for research purposes and those denying this consent were excluded. All patient information is anonymized and kept strictly confidential. The whole research protocol received review and approval from the Institutional Review Board and Ethics Committee of Henan Provincial People's Hospital (approval number: SYSZ-LL-2021091501).

Endometrial Preparation Protocols

Natural Cycles (NCs), Including Modified Cycles

Patients undergoing the natural cycle (NC) preparation protocol commenced monitoring follicular proliferation and endometrial development via transvaginal ultrasound from the tenth to twelfth day of the menstrual cycle until the occurrence of ovulation or luteinization; the detection of LH in urine or blood may be utilized to facilitate diagnosis when deemed necessary. Considering the individual variability in the temporal sequence of luteal peaks, a dosage of 10,000 IU of human chorionic gonadotropin (HCG) was administered to induce ovulation when serum luteinizing hormone level fell below 20 IU/L. FET was conducted three days post-ovulation for cleavage stage embryos or five days post-ovulation for blastocysts.

HRT Cycles

In hormone replacement therapy (HRT) cycles, beginning on cycle day three, estradiol valerate (Progynova; Bayer Schering Pharma AG, Berlin, Germany) was administered via the oral route at a daily dosage of 4 to 8 mg. Transvaginal ultrasound was conducted between the 10th and 12th days of medication to assess endometrial thickness. Progesterone was administered to facilitate endometrial transformation once the EMT reached 7 mm or approached the maximum thickness observed in all previous fresh and FET cycle. FET was subsequently conducted after four days of progesterone therapy for cleavage stage embryos or six days for blastocysts.

GnRH-A-HRT Cycles

In the GnRH-A–HRT cohort, participants underwent a single injection of 3.75 mg of long-acting triptorelin acetate (Diphereline; Bayer Schering Pharma AG, Germany) on days 1 through 4 of the menstrual cycle. The normative criteria for pituitary downregulation are delineated as follows: EMT < 5 mm, luteinizing hormone (LH) < 5 IU/L, estrogen (E2) < 50 pg/mL, and the absence of large cysts or follicles. After approximately thirty days, estrogenic stimulation was administered in accordance with the HRT cycles.

Luteal Phase Support

Luteal phase support protocols for the GnRH-A-HRT cohort and HRT cohort comprised a sustained-release vaginal gel administered at a dosage of 90 mg/day (Crinone; Merck, Germany) alongside oral delivery of progesterone tablets (Duphaston; Abbott Healthcare Products B.V., Netherlands) at a dosage of 40 mg/day. Estradiol valerate continued to be administered as previously, and the dosage of progesterone remained unchanged in the NC cohort. Following FET, all participants administered luteal phase support up to day 14, when serum β-HCG concentrations were assessed. In the event of a positive result, hormone administration was maintained until 12 weeks of gestation.

Outcome Measures

At 14 days post-embryo transfer, serum β-HCG concentrations were evaluated. Transvaginal ultrasonography was conducted in participants with positive β-HCG results at 28 and 35 days following embryo transfer. Obstetric and neonatal outcomes were assessed through telephone interviews. The primary outcome was the LBR, while secondary outcomes included the CPR, early miscarriage rate, and ectopic pregnancy rate. The LBR is characterized as the number of deliveries yielding at least one live birth, quantified per one hundred embryo transfer cycles.[18] Clinical pregnancy is characterized as a confirmed pregnancy through ultrasonographic visualization of gestational sacs; this includes both intrauterine pregnancies and ectopic pregnancies. The CPR is characterized as the number of clinical pregnancies per one hundred embryo transfer cycles. Early miscarriage is defined as spontaneous miscarriage occurring during the first 12 weeks of gestation following verification of pregnancy, and the early miscarriage rate is determined by calculating the ratio of early miscarriages to the total number of clinical pregnancy cycles. The ectopic pregnancy rate is calculated as the ratio of ectopic pregnancies to the total number of clinical pregnancy cycles.

Statistical Methods

Statistical analyses were performed utilizing IBM SPSS Statistics version 25 (v. 25.0; International Business Machines Corporation, Armonk, NY, USA). Initially, we employed the imputation approach method to address missing data. Subsequently, a normality test was performed on continuous variables. If the data satisfied the normality assumption, a T-test was employed, and results were reported as mean ± standard deviation. In cases where normal distribution was not observed, the Kruskal–Wallis H-test was utilized, with results reported as median and interquartile range. The Chi-square test was applied to nominal variables, with percentages (n) used for statistical description. Multivariate regression examinations were conducted to elucidate factors correlated with the LBR. Odds ratios (ORs) and 95% confidence intervals (CIs) were computed to elucidate the association between endometrial preparation protocols and LBR, after adjusting for variables in the multivariate regression model. A p-value below 0.05 was regarded as statistically significant.

Finally, subgroup analyses were pre-planned for the number of fibroids (one versus two or more), and dimension (diameter of the larger fibroid <3 versus 3–5 cm). This cut-off was based on the results of previous studies.[19,20]

Results

Baseline Characteristics

This retrospective research ultimately included 360 participants, who were categorized into three groups: the GnRH-A–HRT group (n = 84), the HRT group (n = 180), and the NC group (n = 96). The baseline characteristics of the study participants are presented in Table 1.

Significant differences were observed in the maximum fibroid diameter, with measurements of 1.90 (1.13, 3.00) cm, 2.00 (1.50, 3.00) cm, and 2.85 (1.60, 4.20) cm respectively (P < 0.001). Nevertheless, no statistically significant variances were detected in female age, body mass index (BMI), duration of infertility, type of infertility, method of fertilization, etiology, anti-Müllerian hormone (AMH), basal follicle-stimulating hormone (FSH), basal luteinizing hormone (LH),

Table 1 Baseline Characteristics and Embryo Transfer Variables of Study Participants

	NC	HRT	GnRH-agonist-HRT	P value
Number of cycles	96	180	84	
Female age (y) *(M, P25, P75)*	35(32,38)	37(33,41)	35.50(33,38)	0.276
BMI (kg/m2) *(M, P25, P75)*	22.67(20.70,24.58)	22.62(21.10,24.80)	22.68(21.10,25.39)	0.181
Infertility duration years (y) *(M, P25, P75)*	2.50(1.63,4.00)	2.50(1.00,5.00)	3.00(1.50,4.00)	0.612
Infertility type (n, %)				
Primary sterility	20(20.8%)	60(33.3%)	28(33.3%)	0.073
Secondary sterility	76(79.2%)	120(66.7%)	56(66.7%)	
Fertilization type (n, %)				
IVF	84(87.5%)	144(80.0%)	68(81.0%)	0.282
ICSI	12(12.5%)	36(20.0%)	16(19.0%)	
Aetiology, n (%)				
Tubal factor	76(79.2%)	156(86.7%)	60(71.5%)	0.527
Ovulatory factor	10(10.4%)	16(8.9%)	10(11.9%)	
Male factor	2(2.1%)	6(3.3%)	8(9.5%)	
Others	8(8.3%)	2(1.1%)	6(7.1%)	
AFC (n) *(M, P25, P75)*	7.50(4.00,11.75)	7.00(4.00,11.00)	8.00(5.00,13.00)	0.445
AMH (ng/mL) *(M, P25, P75)*	1.39(0.75,2.95)	1.38(0.61,3.54)	2.21(0.92,3.21)	0.297
Basal FSH (mIU/mL) *(M, P25, P75)*	6.39(5.53,7.61)	6.76(5.57,8.54)	6.54(5.89,7.95)	0.149
Basal LH (mIU/mL) *(M, P25, P75)*	4.21(2.60,6.45)	4.38(3.13,5.96)	4.53(3.37,5.97)	0.814
Number of eggs (n) *(M, P25, P75)*	6(5,11)	7(3,12)	8(6,13)	0.780
Endometrial thickness on the day of transfer (cm) *(M, P25, P75)*	6.10(5.30,7.00)	6.20(5.70,7.00)	6.50(6.00,6.90)	0.168
No. of transferred embryos (n, %)				
One embryo transferred	52(54.2%)	86(47.8%)	38(45.2%)	0.167
Two embryos transferred	44(45.8%)	94(52.2%)	44(52.4%)	
Three embryos transferred	0	0	2(2.4%)	
Type of embryo transferred (n, %)				
Cleavage stage embryo	42(43.8%)	88(48.9%)	42(50%)	0.644
Blastocyst	54(56.3%)	91(51.1%)	42(50%)	
Maximum fibroid diameter (cm) *(M, P25, P75)*	1.90(1.13,3.00)	2.00(1.50,3.00)	2.85(1.60,4.20)	<0.001
Quantity of fibroids (n, %)				
1	54(56.3%)	116(64.4%)	58(69%)	0.156
2	18(18.8%)	38(21.1%)	12(14.3%)	
≥3	24(25.0%)	26(14.4%)	14(16.7%)	

Abbreviations: NC, natural cycle; HRT, hormone replacement therapy; GnRH-A-HRT, GnRH-A combined with hormone replacement therapy; BMI, Body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone (LH) IVF In vitro fertilization; ICSI Intracytoplasmic sperm injection; GnRH, gonadotropin-releasing hormone; AMH, anti-Müllerian hormone (AMH); AFC, antral follicle count.

Table 2 Pregnancy Outcome After Embryo Transfer in Patients with Thin Endometrium and Intramural Fibroids

	NC	HRT	GnRH-agonist-HRT	P value
Number of cycles	96	180	84	
LBR	20.8% (20/96)	6.7% (12/180)	23.8% (20/84)	<0.001
CPR	29.2% (28/96)	14.4% (26/180)	35.7% (30/84)	<0.001
Early miscarriage rate (%)	14.3% (4/28)	38.5% (10/26)	33.3% (10/30)	0.129
Ectopic pregnancy rate (%)	0 (0/28)	7.69% (2/26)	0 (0/30)	0.087

Abbreviations: NC, natural cycle; HRT, hormone replacement therapy; GnRH-A-HRT, GnRH-A combined with hormone replacement therapy; LBR, live birth rate; CPR, clinical pregnancy rate.

antral follicle count (AFC), number of oocytes retrieved, endometrial thickness on the day of transfer, number of embryos transferred, type of embryo transferred, and the quantity of fibroids among the three cohorts.

Clinical Outcomes

As presented in Table 2, the differences in LBR (20.8% vs 6.7% vs 23.8%, $p < 0.001$) and CPR (29.2% vs 14.4% vs 35.7%, $p < 0.001$) were statistically significant among the three endometrial preparation protocols. Conversely, the early miscarriage rate (14.3% vs 38.5% vs 33.3%, $p = 0.129$) and ectopic pregnancy rate (0% vs 0.7 0.69% vs 0.0%, $p = 0$ 0.087) did not demonstrate any significant differences among these preparations.

Multivariable Logistic Regression Analyses

A multivariable logistic regression examination was conducted with the LBR as the dependent factors, and female age, BMI, infertility type, aetiology, AMH, basal FSH, AFC, number of transferred embryos, endometrial thickness on the day of transfer, type of embryo transferred, maximum fibroid diameter, quantity of fibroids, and endometrial preparation regimens as independent factors. The logistic regression analysis indicated that, among participants with thin endometrium and intramural fibroids, the sole independent variable predictive of LBR was the endometrial preparation regimen. Specifically, when compared to the GnRH-A–HRT group, designated as the reference cohort, the LBR was significantly reduced in both the NC group (adjusted OR: 0.524, 95% CI: 0.457–0.956, $p = 0.023$) and the HRT group (adjusted OR: 0.269, 95% CI: 0.114–0.637, $p = 0.003$) (Table 3).

Table 3 Multivariate Logistic Regression Analysis of Factors Associated with LBR

Clinical indicators	OR (95% CI)	P-value
Female age(y)	0.996(0.920–1.077)	0.911
BMI	0.936(0.830–1.055)	0.279
AMH	0.986(0.770–1.262)	0.909
AFC	1.031(0.938–1.132)	0.529
Basal FSH	0.851(0.725–0.951)	0.059
Fertilization type		
IVF	Ref.	
ICSI	0.755(0.291–1.954)	0.562
Endometrial thickness on the day of transfer (mm)	1.459(0.963–2.211)	0.075
Number of transferred embryos	1.245(0.560–2.772)	0.591
Type of embryo transferred		
Cleavage-stage embryo	Ref.	
Blastocyst	0.377(0.141–1.006)	0.051
Maximum fibroid diameter	1.128(0.907–1.403)	0.278

(*Continued*)

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Table 3 (Continued).

Clinical indicators	OR (95% CI)	P-value
Quantity of fibroids		
1	Ref.	
2	0.668(0.271–1.645)	0.380
≥3	0.584(0.187–1.825)	0.355
Endometrial preparation regimens		
GnRH agonist-HRT	Ref.	
HRT	0.269(0.114–0.637)	0.003
NC	0.524(0.457–0.956)	0.023

Notes: Adjusted by female age, BMI, AMH, AFC, basal FSH, fertilization type, endometrial thickness on the day of transfer; number of transferred embryos, type of embryo transferred, maximum fibroid diameter, quantity of fibroids, and Endometrial preparation regimens.

Abbreviations: NC, natural cycle; HRT, hormone replacement therapy; GnRH-A-HRT, GnRH-A combined with hormone replacement therapy; BMI, Body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone (LH) IVF In vitro fertilization; ICSI Intracytoplasmic sperm injection; GnRH, gonadotropin-releasing hormone; AMH, anti-Müllerian hormone (AMH); AFC, antral follicle count; OR, odds ratio; CI, confidence interval.

Subgroup Analyses

The further subgroup analyses according to number (one versus two or more) and dimension fibroids (<3 versus 3–5 cm) are showed in Table 4. The baseline characteristics of the different subgroups are presented in Supplemental Table 1-4. Among three protocols, significant differences were observed in the number of eggs in the subgroup of dimension <3cm; basal FSH in the subgroup with a single fibroid (N=1), and the number of transferred embryos in the subgroup with two or more fibroids (N≥2). Nevertheless, no statistically significant variances were detected in other clinical indicators in the four subgroups among three protocols. In all four subgroups, participants adhering to the GnRH-A–HRT protocol demonstrated improved LBR and CPR, showing statistically significant differences (Table 4).

In all these four subgroups, a multivariable logistic regression examination was conducted respectively with the LBR as the dependent factors, and female age, BMI, infertility type, aetiology, AMH, basal FSH, AFC, number of transferred embryos, endometrial thickness on the day of transfer, type of embryo transferred, maximum fibroid diameter, quantity of fibroids, and endometrial preparation regimens as independent factors (Supplemental Table 5). The logistic regression analysis indicated that, among participants in all four subgroups, the endometrial preparation regimen was the independent variable predictive of LBR. Additionally, BMI was the independent variable predictive of LBR both in fibroid dimensions <3 cm subgroup and in the subgroup of two or more fibroids (N≥2). AFC was also the independent variable predictive of LBR in the subgroup of two or more fibroids (N≥2). In the fibroid dimensions of 3–5 cm subgroup, endometrial thickness on the day of transfer was also an independent predictor of LBR.

Table 4 Subgroup Analyses According to Number and Dimension of Intramural Fibroids

Group	Pregnancy Outcome	NC	HRT	GnRH-agonist-HRT	P value
Maximum fibroid diameter					
<3cm	LBR	24.4% (18/82)	7.9% (11/140)	30.0% (15/50)	<0.001
	CPR	31.7% (26/82)	15.0% (21/140)	36.0% (18/50)	0.001
3–5cm	LBR	14.3% (2/14)	2.5% (1/40)	14.7% (5/34)	0.044
	CPR	14.3% (2/14)	12.5% (5/40)	35.3% (12/34)	<0.001
Quantity of Fibroids					
N=1	LBR	22.2% (12/54)	8.6% (10/116)	24.1% (14/58)	0.018
	CPR	31.5% (17/54)	17.2% (20/116)	36.2% (21/58)	<0.001
N≥2	LBR	19.0% (8/42)	3.1% (2/64)	23.1% (6/26)	0.003
	CPR	26.2% (11/42)	9.4% (6/64)	34.6% (9/26)	0.015

Abbreviations: NC, natural cycle; HRT, hormone replacement therapy; GnRH-A-HRT, GnRH-A combined with hormone replacement therapy; LBR, live birth rate; CPR, clinical pregnancy rate.

Discussion

In this extensive retrospective study, participants with thin endometrium and intramural fibroids demonstrated improved LBR and CPR following the GnRH-A–HRT protocol, showing statistically significant differences. However, no significant differences were observed in early miscarriage rates or ectopic pregnancy rates among the three endometrial preparation schemes. Furthermore, the application of the GnRH-A–HRT protocol resulted in thicker EMT, indicating that prior conditioning may be advantageous for individuals with a thin endometrium. Besides, when conducting subgroup analyses according to number and dimension of intramural fibroids, the efficacy of the GnRH-A–HRT regimen remains evident.

With the ongoing advancements of ART and laboratory embryo culture techniques, the quality of embryos has been progressively enhanced, consequently, endometrial function and receptivity have emerged as critical determinants influencing embryo implantation. Several tools for endometrial evaluation have been investigated,[21] among them, ultrasound assessment of EMT is widely used as a routine prognosis factor for pregnancy. An EMT of 7 mm has been widely recognized as the threshold for defining thin endometrium in FET cycles.[22] Thin endometrium is not only associated with reduced pregnancy rates but also appears to correlate with adverse perinatal outcomes, miscarriages, and abnormal placentation.[3,4,23,24] The cause of thin endometrium has not yet been understood, but adhesions caused by various intrauterine manipulation (such as induced abortion) is considered to be the primary cause.[25] Recently, numbers of treatments have been proposed to enhance EMT and optimize pregnancy rates of thin endometrium, including the administration of additional estrogen and GnRH agonists, hysteroscopic adhesiolysis followed by various adjunctive therapies, vitamin E supplementation, low-dose aspirin therapy, stem cell regenerative approaches, among others. However, research on thin endometrium remains in the exploratory stages, and the therapeutic effects of these interventions have yet to be fully delineated.[26] In ART, the preference for FET over fresh embryo transfer is predominantly observed among most reproductive centers and patients, primarily due to concerns regarding low endometrial thickness. As of now, numerous researchers have performed comprehensive analyses and comparative assessments of reproductive outcomes in patients with thin endometrium undergoing endometrial preparation for FET across different populations using various preparation protocols.[27,28] However, the existing evidence remains controversial and insufficient.[29]

Uterine fibroids represent the most prevalent benign neoplasms among females of reproductive age, with estimates suggesting that the likelihood of developing uterine fibroids may increase to 75% by the age of 50.[30] The potential mechanisms by which uterine fibroids adversely affect fertility, as studied to date, encompass altered uterine vascular perfusion dynamics, endometrial function, myometrial contractile function, gamete migration, and myometrial/endometrial genomic expression patterns.[31] A considerable number of studies have investigated the impact of GnRH-A on the reduction of both uterine and fibroid volume prior to myomectomy, as well as its role in delaying the recurrence of multiple uterine fibroids postoperatively.[12] Currently, GnRH-A downregulation protocols are frequently employed in individuals with endometriosis, adenomyosis, PCOS, reduced ovarian reserve, and those experiencing RIF. However, the therapeutic effect of GnRH-A pretreatment prior to HRT in patients with uterine fibroids remains contentious regarding pregnancy outcomes.[8]

To the best of our understanding, this represents the first investigation into whether pretreatment with GnRH agonists could improve pregnancy outcomes in patients with intramural fibroids and a thin endometrium, compared to the NC group and the HRT group. In our study, the GnRH-A–HRT protocol exhibited a significant benefit in enhancing both the LBR and CPR. This beneficial effect is consistently observed across the four subgroups divided according to number (one versus two or more) and dimension fibroids (<3 versus 3–5 cm). Additionally, following the administration of GnRH-A, endometrial thickness was improved compared to both the NC group and the HRT group; nevertheless, this difference did not achieve statistical significance. This observation aligns with a previous retrospective cohort analysis that indicated GnRH-A pre-treatment can improve EMT on the day of progesterone therapy initiation and enhance the LBR.[8] In another extensive analysis, the GnRH-A–HRT protocol was additionally correlated with a higher LBR compared to the NC protocol in frozen blastocyst-stage transfer cycles.[32] GnRH-A are synthetic peptides that are structurally analogous to natural GnRH, which is secreted in a pulsatile pattern by the hypothalamus. When administered chronically, they inhibit normal pituitary-gonadal function and are widely utilized in routine reproductive medicine

practice.[33] Numerous researchers believe that GnRH-A may confer beneficial effects related to enhanced endometrial receptivity during embryo implantation. Recent investigations into molecular mechanisms have demonstrated that the administration of GnRH-A can positively influence endometrial receptivity by modulating the expression of endometrial adhesion molecules, genetic modifications, and implantation biomarkers, including HOXA10, MEIS, and LIF, which are recognized for their roles in promoting uterine ontogenesis and enhancing endometrial receptivity.[34,35] Moreover, a separate study in mice demonstrated that GnRH-A partially reinstated the expression of endometrial integrin β3 and enhanced endometrial receptivity.[35]

To eliminate confounding factors that may influence the endometrium, we omitted participants diagnosed with adenomyosis, endometriosis, PCOS, RIF, RSA, and uterine malformations. As postulated, maternal age, infertility duration, AMH, basal FSH, AFC, BMI, infertility type, and aetiology were commensurable throughout the three groups. The application of multivariate logistic regression examination and adjusted marginal means (95% confidence intervals) concerning the LBR and CPR further facilitated the elimination of confounding factors. The multivariate logistic regression model indicates that the modality of endometrial preparation is a significant factor affecting both the LBR and CPR. Furthermore, the intima thickness is significantly improved in the GnRH-A–HRT group. Although the precise mechanisms remain unclear, they may be attributed to endometrial thickening, which can prolong the pregnancy duration and increase the likelihood of conception and live birth.[36] Furthermore, the perturbation of the consecutive menstrual cycle engendered by the extended down-regulation of the pituitary gland might facilitate the full functionality of the hormone-sensitive system.[37] However, this conclusion needs to be confirmed.

Estrogen plays a crucial role in endometrial growth.[38] Consequently, various hormonal strategies have been developed to address thin endometrium. Similarly, the findings of our study demonstrated that HRT treatment enhanced endometrial thickness. However, for individuals with thin endometrium and intramural fibroids, the pregnancy outcomes in the HRT group were not only significantly worse than those in the GnRH-A group but also inferior to those observed in the NC group. In this study, although the LBR and CPR were lower in the NC group compared to the GnRH-A-HRT group, the early miscarriage rate was also reduced in the NC group, albeit without reaching statistical significance. The NC protocol is among the most commonly employed and widely utilized FET protocols in ART.[39] A meta-analysis demonstrates that the clinical CPR in patients undergoing the NC protocol is significantly superior to that of those in the HRT group. The selection of an appropriate endometrial preparation strategy should be customized to align with each patient's unique circumstances.[40] In the NC protocol, the formation of a corpus luteum following natural ovulation results in an endometrium that more closely resembles the conditions of natural pregnancy, thereby enhancing endometrial receptivity.[41] The corpus luteum synthesizes progesterone and estrogen, in addition to vasoactive agents such as relaxin, angiogenic metabolites, and vascular endothelial growth factor (VEGF) derived from estrogen. The HRT protocol is correlated with an insufficiency of these vasoactive products compared to the NC protocol, which involves the presence of a corpus luteum.[41,42] Furthermore, within the HRT protocol, once the EMT reaches the specified criterion of approximately 0.8 centimeters, luteal supplementation is typically initiated rather than prolonging the proliferative phase to attain increased thickness. In contrast, during a natural protocol, the endometrium generally attains optimal thickness in conjunction with the development and rupture of the dominant follicle.

This investigation presents three notable advantages. Firstly, to the best of our understanding, this research is the inaugural investigation to ascertain which endometrial preparation regimen may yield superior reproductive outcomes for patients of thin endometrium with uterine fibroid. This study possesses significant clinical implications and offers valuable insights for the selection of individualized frozen embryo transfer protocols. Secondly, the substantial sample size of this single-center investigation, where clinical and IVF laboratory procedures were consistently standardized, alleviated potential biases and potentiated statistical power. Thirdly, we delineated rigorous exclusion and inclusion criteria throughout the screening of the study cohort, thereby eliminating disorders that might potentially impinge upon reproductive outcomes and the integrity of the endometrium and uterine cavity, such as adenomyosis, endometriosis, and uterine malformations. Moreover, patients diagnosed with RSA and RIF were also precluded.

This study also presents certain limitations. Primarily, this investigation is a retrospective cohort study. As with any retrospective analysis, certain inherent limitations are unavoidable. It is also noteworthy that some participants with

a thin endometrium underwent fresh embryo transfer, whereas others opted to cancel the transfer. This discrepancy may have introduced bias and consequently might not accurately reflect the true impact of varying endometrial preparation protocols. Furthermore, the data regarding the maximum fibroid diameter were obtained prior to the initiation of a treatment cycle. No re-assessment of uterine fibroid size was conducted on the day of transfer, thereby precluding the observation of the effects of the three endometrial preparation regimens. Nevertheless, this does not affect the conclusion regarding the positive impact of the GnRH-A-HRT protocol on patients' LBR, as the maximum fibroid diameter was significantly larger in the GnRH-A group compared to the other two groups; however, this finding underscores the need for additional multicenter, large-scale prospective studies to further validate and corroborate our results.

Conclusions

Our research indicates that, compared to NC and HRT protocol, significantly improved reproductive outcomes were observed in the GnRH-A–HRT group. These findings also provide valuable insights for further exploration of the underlying mechanisms by which the GnRH-A-HRT protocol enhances reproductive outcomes in patients of thin endometrium with intramural fibroids.

Data Sharing Statement

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Informed Consent Statement

Not applicable.

Consent for Publication

Not Applicable.

Institutional Review Board Statement

This study was conducted in accordance with the Declaration of Helsinki, as revised in 1983. This study was reviewed and approved by the Institutional Review Board and Ethics Committee of Henan Provincial People's Hospital, China.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest.

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