

Original Research

Impact of Pretreatment With a Gonadotropin-Releasing Hormone Agonist and Letrozole on *In Vitro* Fertilization Outcomes in Normal Responders With Ovarian Endometrioma

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Abstract

Background: The optimal pretreatment strategy for patients with ovarian endometrioma (OEM) undergoing *in vitro* fertilization (IVF) remains controversial, particularly among those with a normal ovarian response. Letrozole (LE) has been proposed to modulate the ovarian hormonal environment and improve ovarian stimulation outcomes. However, its clinical value in this patient population has not been fully established. We assessed the clinical value of pretreatment with LE in combination with a gonadotropin-releasing hormone agonist (GnRHa) in patients with OEM and normal ovarian response undergoing IVF. **Methods:** In this retrospective study, 71 patients with OEM and normal ovarian response who underwent IVF-embryo transfer (IVF-ET) were initially enrolled and assigned to the GnRHa + LE group (n = 36) or the GnRHa group (n = 35). After one patient was excluded from each group, 35 patients in the GnRHa + LE group and 34 patients in the GnRHa group were included in the final transfer-cycle analysis. During controlled ovarian stimulation, patients received pretreatment with GnRHa (3.75 mg) either alone or in combination with LE (2.5 mg) and were accordingly allocated to the GnRHa group or the GnRHa + LE group. Between-group differences were evaluated regarding baseline characteristics, ovarian stimulation profiles, and reproductive outcomes. **Results:** The total gonadotropin dose was significantly lower in the GnRHa + LE group and was accompanied by reduced serum estradiol levels on the trigger day ($p < 0.01$). This group also demonstrated increased numbers of retrieved and metaphase II oocytes (all $p < 0.01$), as well as improved fertilization rates ($p = 0.02$) and day-3 high-quality embryo rates ($p < 0.05$). Stimulation duration, cleavage rate, progesterone levels, embryo transfer number, endometrial thickness, and fresh embryo transfer cancellation rates did not differ significantly between groups (all $p > 0.05$). The clinical pregnancy rate was significantly higher in the GnRHa + LE group ($p = 0.04$). The implantation rate was significantly higher in the GnRHa + LE group than in the GnRHa group ($p = 0.04$), whereas the early miscarriage rate was comparable between the two groups ($p = 0.82$). Reduction in maximal ovarian-endometriotic-cyst diameter was significantly greater in the GnRHa + LE group ($p = 0.04$). **Conclusions:** Combined pretreatment with LE and GnRHa may enhance ovarian response efficiency and improve IVF-ET outcomes among women with OEM who exhibit a normal ovarian response.

Keywords: gonadotropin-releasing hormone agonist; infertility; *in vitro* fertilization; letrozole; normal ovarian response; ovarian endometrioma

1. Introduction

Endometriosis is a chronic estrogen-dependent gynecological disorder characterized by the ectopic presence of functional endometrial-like tissue outside the uterine cavity, and is commonly associated with dysmenorrhea, pelvic pain, infertility, and impaired organ function. It affects approximately 10%–20% of women of reproductive age, with up to 50% experiencing infertility [1]. Moreover, beyond its clinical manifestations, endometriosis is associated with a compromised reproductive microenvironment, including chronic inflammation, immune dysregulation, and impaired endometrial receptivity, which may adversely affect assisted reproductive technology outcomes.

Ovarian endometrioma (OEM) is the most common subtype among infertile women, with approximately 17%–44% of patients undergoing assisted reproductive treatment, including *in vitro* fertilization and embryo transfer (IVF-ET), being affected. Ovarian endometriomas may ad-

versely affect fertility and reproductive outcomes in women with endometriosis [2]. In addition, OEM has been reported to adversely affect IVF outcomes by decreasing oocyte quality, reducing fertilization rates, impairing embryo development, and increasing the risk of implantation failure and early pregnancy loss.

Gonadotropin-releasing hormone agonists (GnRHa) remain a key medical therapy for endometriosis and are widely integrated into IVF-ET protocols. Sallam et al. [3] reported that 3–6 months of GnRHa pretreatment increased clinical pregnancy rates in women with endometriosis by nearly fourfold. Long-acting GnRHa before embryo transfer has also been shown to lower early miscarriage rates [4]. However, evidence regarding GnRHa-induced reduction of ovarian endometriotic cysts is inconsistent, likely due to insufficient suppression of extragonadal estrogen production [5].



Mechanistically, GnRHa treatment initially induces a transient flare-up effect followed by downregulation of pituitary gonadotropin secretion, leading to profound hypoeestrogenism and suppression of endometriotic lesion activity. This hypoeestrogenic state contributes to reduced lesion proliferation, decreased inflammatory cytokine production, and improved pelvic environment [1,5].

Letrozole (LE), a selective aromatase inhibitor, suppresses both intra- and extra-ovarian estrogen biosynthesis, thereby inhibiting estrogen-dependent lesion growth. In one study, three months of high-dose LE (5 mg/day) combined with a progestin reduced mean cyst diameter by about 50% and cyst volume by 75% [6]. At the molecular level, LE inhibits the aromatase enzyme responsible for the peripheral conversion of androgens to estrogens, thereby reducing both systemic and local estrogen concentrations. This dual suppression is particularly relevant in endometriosis, where ectopic lesions exhibit aberrant aromatase expression and local estrogen production [6].

Given the complementary mechanisms of GnRHa and LE, we hypothesized that combined pretreatment would enhance IVF outcomes in OEM patients. Specifically, whereas GnRHa suppresses ovarian estrogen production via pituitary downregulation, LE further inhibits peripheral and local estrogen synthesis, potentially resulting in more complete estrogen deprivation and improved control of endometriotic activity. This study therefore evaluated the clinical value of GnRHa plus LE pretreatment in normal-responder women with OEM who were undergoing IVF-ET.

2. Materials and Methods

2.1 Study Sample

A total of 125 patients with OEMs and normal ovarian response who underwent IVF-ET at the Reproductive Center of Xuzhou Central Hospital between March 2023 and March 2025 were initially assessed for eligibility. OEM was not considered the sole cause of infertility in all cases; instead, all patients had a confirmed diagnosis of OEM, although additional infertility factors (such as tubal or male factors) could coexist. Of these, 54 patients were excluded for the following reasons: age <23 or >35 years ($n = 21$), failure to meet the predefined criteria for normal ovarian response ($n = 8$), prior surgery for endometriosis ($n = 9$), uterine abnormalities, endometrial disorders, or hydrosalpinx potentially affecting implantation ($n = 11$), and total motile sperm count $<5 \times 10^6$ in the male partner ($n = 5$).

Ultimately, 71 patients were enrolled and assigned to either the GnRHa + LE group ($n = 36$) or the GnRHa group ($n = 35$), according to whether LE pretreatment was used. One patient in each group was subsequently excluded (due to elevated progesterone level on trigger day in the GnRHa + LE group and personal reasons in the GnRHa group), leaving 35 and 34 patients, respectively, for the final analysis (Fig. 1).

2.2 Inclusion Criteria

The inclusion criteria were: (1) age between 23 and 35 years; (2) diagnosed with OEMs only; other subtypes of endometriosis (e.g., deep infiltrating endometriosis or peritoneal endometriosis) were not included. (3) no previous surgical history related to endometriosis; OEMs were diagnosed by experienced clinicians using transvaginal ultrasonography, according to the International Ovarian Tumor Analysis (IOTA) consensus criteria [7], characterized by cysts with regular margins and homogeneous low-level “ground-glass” echogenicity; (4) normal ovarian response meeting the following criteria [8]: (a) a basal follicle-stimulating hormone (FSH) level below 10 IU/L; (b) bilateral antral follicle count (AFC) between 7 and 14; (c) anti-Müllerian hormone (AMH) between 1.0 ng/mL and 4.0 ng/mL; (d) no history of ovarian-stimulation-cycle cancellation prior to oocyte retrieval due to poor or excessive ovarian response.

2.3 Exclusion Criteria

The exclusion criteria were: (1) either partner presenting with chromosomal abnormalities; (2) uterine abnormalities (e.g., congenital malformations, adenomyosis, or leiomyoma), endometrial lesions (e.g., endometritis, polyps, or intrauterine adhesions), or hydrosalpinx that may affect embryo implantation; (3) endocrine, immunologic, or metabolic disorders such as thyroid disease, diabetes mellitus, hyperprolactinemia, or polycystic ovary syndrome; (4) male factor infertility, particularly severe male factor defined as total motile sperm count $<5 \times 10^6$ in the male partner [9], thereby minimizing the potential confounding effect of male-related factors on IVF outcomes; (5) severe systemic diseases, including major organ dysfunction, malignancy, or a history of thromboembolic events; (6) poor ovarian responders and high ovarian responders, were excluded to ensure a homogeneous population of normal ovarian responders.

This retrospective study was approved by the Ethics Committee of our hospital. The requirement for written informed consent was waived due to the retrospective nature of the study. Ethical approval was obtained after completion of patient follow-up, and all data were anonymized prior to analysis.

2.4 Controlled Ovarian Stimulation Protocols

Patients were not randomly assigned in this retrospective study; instead, the allocation to each treatment group was based on clinical decision-making, considering physician preference and patient-specific conditions at the time of treatment. Two different controlled ovarian stimulation protocols were implemented as described below:

GnRHa group ($n = 35$): On menstrual cycle days 1–3, patients received an injection of triptorelin acetate 3.75 mg, s.c. (Decapeptyl, Ferring Pharmaceuticals SA, Saint-Prex, Switzerland). The injection was repeated after 30 days for a total of two doses.

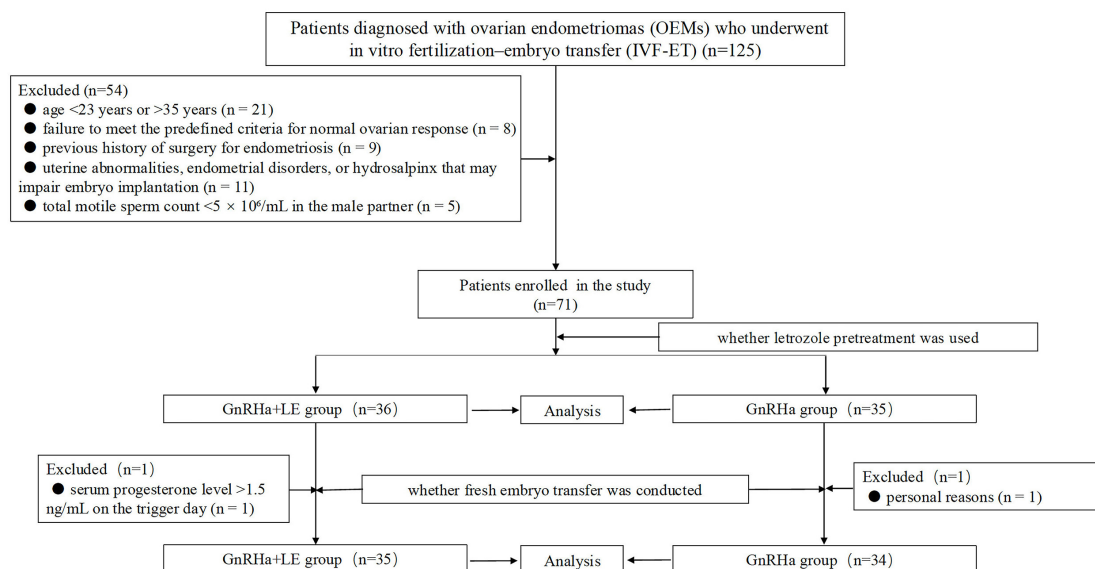


Fig. 1. Flow diagram of the study. GnRHa, gonadotropin-releasing hormone agonist; LE, letrozole.

GnRHa + LE group (n = 36): On menstrual cycle days 1–3, patients underwent the same GnRHa pretreatment as described above and received 2.5 mg LE (Jiangsu Hengrui Pharmaceuticals Co., Ltd., 231205KL, Lianyungang, Jiangsu, China) orally once daily for five consecutive days.

Thirty days after the last GnRHa injection, vaginal ultrasonography was performed to assess antral follicle number and size. Serum concentrations of fFSH, luteinizing hormone (LH), and estradiol (E_2) were assessed. Pituitary downregulation [10] was defined as the presence of follicles with a diameter ≤ 10 mm, accompanied by serum FSH and LH levels below 5 mIU/mL and an E_2 level < 50 pg/mL.

Controlled ovarian stimulation was then initiated with recombinant FSH (r-FSH, Gonal-F, Merck Serono GmbH, BA089041/BA096091, Darmstadt, Germany) at 150–225 IU combined with recombinant LH (r-LH, Luveris, Merck Serono GmbH, AU035478/AU043747, Darmstadt, Germany) at 75 IU daily. Dosages were adjusted according to individual ovarian response. When at least one follicle reached ≥ 18 mm or two follicles reached ≥ 17 mm, ovulation was triggered using recombinant human chorionic gonadotropin (r-hCG, Ovitrelle, Merck Serono, BA091066/BA096099, Modugno, Bari, Italy) 0.25 mg, s.c. Oocyte collection was carried out 36–37 hours after trigger administration, guided by transvaginal ultrasonography.

2.5 Fertilization, Embryo Transfer, and Luteal Support

Semen samples were produced from the male partners by masturbation on the day of oocyte retrieval and collected. All cycles in this study were performed using conventional IVF, as patients with severe male-factor infertility were excluded. Fertilization was assessed by the presence of two pronuclei (2PN) at 16–18 h after insemination. Embryo morphology was assessed according to the Istanbul consensus [11].

Patients in the downregulation group received progesterone (Zhejiang Xianju Pharmaceutical Co., Ltd., FB2439, Xianju County, Taizhou, Zhejiang, China), 60 mg/day i.m., and oral estradiol-dydrogesterone tablets (Femoston, Abbott Biologicals B.V., 370364/374233, Olst, Netherlands; containing 2 mg estradiol and 10 mg dydrogesterone, twice daily) for luteal-phase support.

On day 3 post-fertilization, embryo transfer (ET) was performed under ultrasound guidance, with the number of embryos (one or two) determined according to endometrial thickness, serum hormone profiles, and embryo quality. Remaining viable embryos were cryopreserved for frozen-thawed embryo transfer (FET) if fresh transfer was not carried out.

2.6 Pregnancy Assessment and Follow-Up

Serum β -human chorionic gonadotropin (β -hCG) concentrations were assessed 14 days following ET. Levels ≤ 5 mIU/mL indicated implantation failure. Clinical pregnancy was diagnosed when transvaginal ultrasonography at 28–35 days after ET demonstrated both a gestational sac and fetal cardiac activity.

2.7 Outcome Measures

Baseline characteristics were: age; duration of infertility; body mass index (BMI); AFC; AMH; basal hormone levels (FSH, E_2); and mean maximum diameter of OEMs.

Stimulation and laboratory parameters were: duration and total dose of gonadotropin; number of retrieved oocytes; number of metaphase II (MII) oocytes; fertilization rate; cleavage rate; day-3 high-quality embryo rate; serum E_2 and progesterone levels on trigger day; endometrial thickness; number of embryos transferred; and fresh embryo-transfer-cancellation rate.

Pregnancy outcomes were: mean maximum diameter of OEMs on trigger day; implantation rate; clinical pregnancy rate; and early miscarriage rate.

2.8 Definitions of Indicators

The indicators were defined according to previously published studies [11,12,13].

- The total number of oocytes retrieved referred to all oocytes collected during the oocyte retrieval procedure.

- MII oocytes were defined as mature oocytes characterized by the extrusion of the first polar body.

- The fertilization rate was calculated as the proportion of oocytes successfully fertilized among all MII oocytes.

- The cleavage rate represented the percentage of embryos that underwent cleavage relative to the number of normally fertilized oocytes.

- The proportion of high-quality embryos on day 3 was defined as the number of good-quality embryos divided by the total number of cleaved embryos derived from normally fertilized (2PN) zygotes.

- The cancellation rate of fresh embryo transfer cycles was calculated based on the proportion of cycles in which embryo transfer was not performed among all oocyte retrieval cycles.

- The implantation rate was defined as the ratio of successfully implanted embryos to the total number of embryos transferred.

- The clinical pregnancy rate was calculated as the proportion of cycles resulting in clinical pregnancy among all embryo transfer cycles.

- Early miscarriage rate = (number of early miscarriages ÷ number of clinical pregnancies) × 100%.

2.9 Statistical Analysis

All statistical analyses were carried out with SPSS 19.0 (IBM Corp., Armonk, NY, USA). Continuous variables were summarized as mean ± SD and analyzed using the independent samples *t*-test for between-group comparisons. Before applying the *t*-test, the normality of continuous variables was assessed using the Shapiro–Wilk test, and homogeneity of variances was evaluated using Levene’s test. If the assumptions for the *t*-test were not met, non-parametric tests (Mann–Whitney U, Chi-square, Fisher’s exact) were used, as appropriate. With the actual analyzed sample sizes of 35 and 34, the post hoc statistical power to detect differences in embryos transferred and endometrial thickness was approximately 12.8% and 5.7%, respectively, using a two-tailed, two-sample *t*-test with unequal variances at a significance level of 0.05. A *p*-value ≤ 0.05 was considered statistically significant.

3. Results

3.1 Baseline Characteristics of the Study Population

Baseline demographic and clinical parameters were comparable between the two groups. No significant dif-

ferences were observed in age, duration of infertility, BMI, AFC, AMH, basal FSH, basal E₂ levels, or the mean maximal diameter of OEMs (all *p* > 0.05) (Table 1).

3.2 Ovarian Stimulation Outcomes and Embryological Characteristics

Compared with the GnRHa protocol alone, patients receiving the combined GnRHa + LE regimen required a lower total dose of gonadotropin and exhibited reduced serum E₂ concentrations on the day of trigger (both *p* < 0.05). In contrast, the GnRHa + LE group yielded a greater number of retrieved and MII oocytes, along with improved fertilization outcomes and a higher proportion of high-quality embryos on day 3 (*p* < 0.05 for all comparisons). However, no significant differences were identified between the two groups in the duration of stimulation, cleavage rate, or progesterone level on the trigger day (*p* > 0.05) (Table 2).

3.3 Comparison of Treatment Outcomes and Pregnancy Results

Fresh embryo transfer was carried out in 35 cycles in the GnRHa + LE group and in 34 cycles in the GnRHa group. Baseline transfer characteristics, including the number of embryos transferred, endometrial thickness on the transfer day, and the rate of ET cancellation, were comparable between the two groups (all *p* > 0.05). Compared with the GnRHa group, the GnRHa + LE group demonstrated a significantly higher clinical pregnancy rate, along with an increased implantation rate (all *p* < 0.05) and a comparable incidence of early miscarriage (*p* = 0.82). In addition, the mean maximum diameter of OEMs was significantly smaller in the GnRHa + LE group than in the GnRHa group after pretreatment (*p* < 0.05) (Table 3).

4. Discussion

Endometriosis is an estrogen-dependent disease in which increased estrogen sensitivity and progesterone resistance play critical roles in onset and progression. The disease has an insidious onset and begins to impair fertility even at its early stage, with an average diagnostic delay of approximately 10 years [14]. A study has shown that 22–63% of women with unexplained infertility who undergo laparoscopy are found to have endometriosis [15]. As the disease progresses, ectopic endometrial tissue may invade the pelvic cavity, ovaries, or peritoneum, leading to infertility through multiple mechanisms, including altered coital frequency, ovulatory dysfunction, impaired tubal function, reduced embryo quality, and decreased endometrial receptivity [16]. In addition, endometriosis-associated peritoneal fluid may negatively affect sperm function [17]. An experimental study showed that peritoneal fluid from women with moderate to severe endometriosis can impair sperm motility, potentially due to elevated levels of reactive oxygen species and inflammatory cytokines (e.g., tumor necrosis factor-α [TNF-α], interleukin-6 [IL-6], interleukin-

Table 1. Baseline characteristics of the study groups.

Variable	GnRHa + LE group	GnRHa group	<i>p</i> Value
Cycles (n)	36	35	
Age (years)	30.42 ± 3.55 (23–34)	29.03 ± 2.95 (25–35)	0.08
Type of Infertility, n (%)			0.73
Primary	22 (61.11%)	20 (57.14%)	
Secondary	14 (38.89%)	15 (42.86%)	
Duration of Infertility (years)	3.38 ± 1.00	3.11 ± 1.13	0.31
BMI (kg/m ²)	22.54 ± 1.99	21.90 ± 1.79	0.16
AFC (n)	10.36 ± 2.67	10.71 ± 2.52	0.57
AMH (ng/mL)	2.69 ± 0.70	2.63 ± 0.62	0.70
Basal FSH (mIU/mL)	7.74 ± 1.36	7.34 ± 1.78	0.29
Basal E ₂ (pg/mL)	41.00 (32.00, 65.00)	40.00 (33.00, 60.95)	0.74
Max diameter (mm)	33.67 ± 5.86	32.13 ± 5.07	0.24

Note: BMI, body mass index; AMH, anti-Müllerian hormone; AFC, antral follicle count; Basal FSH, basal follicle-stimulating hormone; Basal E₂, basal estradiol; Max diameter, mean maximum diameter of ovarian endometriomas. Data are presented as mean ± SD, median (IQR), or n (%), as appropriate.

Table 2. Ovarian stimulation outcomes and embryological variables in the two groups [(mean ± SD), %].

Variable	GnRHa + LE group	GnRHa group	<i>p</i> Value
Cycles (n)	36	35	
Stimulation Days	9.97 ± 1.70	9.83 ± 1.62	0.72
Total Gonadotropin Dose (IU)	2182 ± 406.6	2705 ± 416.6	<0.01
Oocytes Retrieved	9.11 ± 2.52	6.97 ± 1.86	<0.01
MII Oocytes	6.75 ± 2.37	4.80 ± 1.55	<0.01
Fertilization Rate (%)	87.24 (212/243)	77.38 (130/168)	0.01
Cleavage Rate (%)	90.09 (191/212)	87.69 (114/130)	0.12
Day 3 high-quality embryo rate (%)	60.73 (116/191)	49.12 (56/114)	<0.05
E ₂ (pg/mL)	1981 ± 556.10	2924 ± 730.50	<0.01
P (ng/mL)	0.83 ± 0.34	0.91 ± 0.18	0.20

Note: MII, metaphase II; E₂, estradiol; P, progesterone.

Table 3. Treatment and pregnancy outcomes in the two groups [(mean ± SD), %].

Variable	GnRHa +LE group	GnRHa group	<i>p</i> Value
Total cycles (n)	36	35	
Fresh embryo transfer cycles (n)	35	34	
Max diameter (mm)	27.14 ± 5.65	29.72 ± 4.68	0.04
Embryos Transferred	1.51 ± 0.51	1.41 ± 0.50	0.40
Endometrial Thickness (mm)	10.86 ± 4.33	10.66 ± 2.01	0.81
Fresh embryo transfer cancellation rate (%)	2.78 (1/36)	2.86 (1/35)	1.00
Implantation Rate (%)	41.51 (22/53)	22.92 (11/48)	0.04
Clinical Pregnancy Rate (%)	57.14 (20/35)	32.35 (11/34)	0.04
Early Miscarriage Rate (%)	15.00 (3/20)	18.18 (2/11)	1.00

Note: Embryos transferred and endometrial thickness were analyzed among fresh embryo transfer cycles (n = 35 in the GnRHa + LE group and n = 34 in the GnRHa group). The fresh embryo transfer cancellation rate was calculated based on all initiated cycles (n = 36 and n = 35, respectively).

8 [IL-8]), which may interfere with sperm capacitation and acrosome reaction [18]. However, the application of assisted reproductive technology enables the majority of patients to attain favorable reproductive outcomes.

OEMs represent an advanced stage of the disease. The cysts can impair ovarian function through mechanical com-

pression and the secretion of inflammatory cytokines, reactive oxygen species, and free iron, thereby reducing ovarian responsiveness, impairing oocyte development, and compromising embryo quality, which ultimately affects IVF-ET outcomes [2]. An *in vitro* study has demonstrated that exposure of parthenogenetically activated oocytes to follic-

ular fluid from patients with OEMs significantly increases oocyte fragmentation and cell death, thereby impairing embryonic development [19].

GnRHa is among the most widely used classes of medications for the treatment of endometriosis. A GnRHa binds to gonadotropin-releasing hormone receptors on the anterior pituitary with high affinity, initially producing a “flare-up effect” that transiently elevates FSH and LH levels and promotes follicular development. With prolonged administration, however, receptor desensitization and downregulation occur, leading to suppression of gonadotropin secretion and reduced estrogen levels. As a result, ectopic endometrial tissue undergoes atrophy due to estrogen deprivation, thereby relieving symptoms and improving fertility in patients with endometriosis [20].

Most studies have demonstrated that pretreatment with GnRHa for 3–6 months before Assisted Reproductive Technology (ART) improves clinical outcomes in patients with advanced endometriosis (stage III–IV), although its benefit in mild disease remains controversial [21,22]. The mechanisms by which GnRHa enhances IVF outcomes are multifactorial. GnRHa reduces the expression of vascular endothelial growth factor, tissue factor, and protease-activated receptor 2 in ectopic endometrium, thereby inhibiting angiogenesis and reducing cell adhesion and invasion [23]. In addition, GnRHa decreases the production of cytotoxic factors in follicular fluid, reduces oxidative stress, and improves oocyte quality. After three months of GnRHa treatment, the concentrations of TNF- α and 8-hydroxy-2'-deoxyguanosine in follicular fluid are significantly reduced, whereas melatonin levels and fertilization rates are higher [24].

GnRHa also improves endometrial receptivity by promoting the development of pinopodes. Li et al. [25] reported that GnRHa pretreatment downregulated miR-124-3p expression and upregulated IL-6, IL-11, Leukemia Inhibitory Factor (LIF), and integrin $\alpha\beta 3$ in the endometrium, which favored early embryo implantation. Furthermore, GnRHa enhanced subendometrial blood perfusion and improved the pelvic inflammatory environment [26]. However, whether GnRHa reduces the size of endometriotic lesions remains unclear. GnRHa primarily suppresses ovarian estrogen production but does not affect estrogen derived from ectopic endometrial tissue, adrenal cortex, or peripheral aromatization, which may explain why some cases are resistant to ovarian suppression [5].

As a third-generation aromatase inhibitor, LE suppresses estrogen production at both central and peripheral levels, thereby establishing a hormonal milieu that more closely resembles physiological conditions. A study has shown that LE effectively reduces serum E₂ levels on the day of trigger and lowers E₂ production per mature follicle without compromising ovulation-induction outcomes, thereby reducing estrogen exposure and potentially limiting disease progression. It is widely considered a relatively safe option for ovulation induction when fertility preservation is

required in individuals with hormone-sensitive gynecologic malignancies [27].

LE has also been shown to improve ovarian response and endometrial receptivity. Among women with poor ovarian response, co-administration of LE with gonadotropins was associated with increased yields of retrieved oocytes, MII oocytes, and high-quality embryos compared with gonadotropin stimulation alone [28]. In hormone replacement embryo-transfer cycles, the addition of LE was linked to increased pregnancy rates, likely due to enhanced endometrial receptivity through molecular mechanisms [29]. An animal study has demonstrated that LE increased the expression of $\alpha\beta 3$ integrin and Homeobox A10 (HOXA10), two markers of endometrial receptivity, in rat endometrium [30].

Combined treatment with GnRHa and LE provided maximal suppression of estrogen production and reduced inflammatory mediators in endometrial tissue. This combined approach has been shown to enhance both the likelihood of achieving a clinical pregnancy and the chance of live birth among women experiencing repeated implantation failure or unexplained endometriosis [31]. Cozzolino et al. [32] reported that GnRHa monotherapy may be insufficient to suppress estrogen secretion in severe adenomyosis, whereas combination therapy with LE was both safe and effective. Similarly, previous studies [33,34] suggest that combining an aromatase inhibitor with GnRHa may improve reproductive outcomes in women with endometriosis. Moreover, since endometriotic epithelial cells harbor oncogenic mutations and endometriosis is linked to a higher risk of ovarian cancer in ART patients [35], the combination of GnRHa and LE may also reduce estrogen exposure and help prevent malignant transformation.

In the present study, GnRHa combined with LE significantly reduced E₂ level on the day of trigger and led to a greater reduction in the mean diameter of OEMs than did GnRHa alone. Notably, patients in the combination group achieved superior reproductive outcomes, including greater oocyte yield, increased numbers of MII oocytes, improved fertilization rates, a higher proportion of good-quality embryos on day 3, as well as enhanced clinical pregnancy rates.

We selected individuals diagnosed with OEM who exhibited a normal ovarian response to minimize the influence of hyper- or hypo-response on the study outcomes. The half-life of LE is 30–60 h, and its optimal dose in ART remains uncertain [36]. Given that higher doses of LE may promote multifollicular development and potentially increase the risk of ovarian hyperstimulation and multiple pregnancy, we adopted a commonly used low-dose regimen of 2.5 mg/day for five days [37]. Although some patients in the combination group experienced mild hypoestrogenic symptoms, no severe adverse reactions were observed.

Limitations

This study had several limitations that should be acknowledged. First, preimplantation genetic testing was not

performed, which may have limited a comprehensive evaluation of embryo quality. Second, the relatively small sample size may have limited the statistical power to detect differences in certain outcomes. As indicated by the post hoc power analysis, the power for several nonsignificant variables, including the number of embryos transferred and endometrial thickness, was less than 20%, indicating a high risk of type II error. Therefore, due to sample size limitations, it is not possible to conclude that there is no difference between the two groups for these outcomes. The absence of statistically significant differences should be interpreted with caution, as true differences may have been missed. Third, the optimal dose and duration of LE in this population remain unclear and require further investigation. Fourth, only patients with OEM were included, which may have restricted the generalizability of the findings. Considering the heterogeneous nature of endometriosis, our results may not be directly applicable to other subtypes, such as deep infiltrating or peritoneal endometriosis. Finally, given the retrospective design and the lack of randomization, potential selection bias cannot be entirely excluded.

5. Conclusions

For patients with OEMs and normal ovarian response, pretreatment with a combination of GnRHa and LE appeared to be more beneficial for reducing cyst size and improving IVF-ET pregnancy outcomes than did pretreatment with GnRHa alone. Large-scale randomized controlled studies are needed in order to validate these findings.

Availability of Data and Materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Author Contributions

YC and YY were involved in data interpretation. JG contributed substantially to the acquisition, curation, verification, and interpretation of the clinical data. QS conceived the study and performed the data analysis. All authors participated in manuscript revision, approved the final version, and agree to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study adhered to the principles of the Declaration of Helsinki and received approval from the Reproductive Medicine Ethics Committee of Xuzhou Central Hospital (Approval No. XZXY-LS-20250624-002). The requirement for written informed consent was waived by the Ethics Committee due to the retrospective nature of the study.

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Conflicts of Interest

The authors declare no conflicts of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/CEOG50028>.

References

- [1] Horne AW, Missmer SA. Pathophysiology, diagnosis, and management of endometriosis. *BMJ*. 2022; 379: e070750. <https://doi.org/10.1136/bmj-2022-070750>
- [2] Hodgson RM, Lee HL, Wang R, Mol BW, Johnson N. Interventions for endometriosis-related infertility: a systematic review and network meta-analysis. *Fertility and Sterility*. 2020; 113: 374–382.e2. <https://doi.org/10.1016/j.fertnstert.2019.09.031>
- [3] Sallam HN, Garcia-Velasco JA, Dias S, Arici A. Long-term pituitary down-regulation before in vitro fertilization (IVF) for women with endometriosis. *The Cochrane Database of Systematic Reviews*. 2006; 2006: CD004635. <https://doi.org/10.1002/14651858.CD004635.pub2>
- [4] Hao H, Li M, Zhang C, Zhang S. The effect of gonadotropin-releasing hormone agonist downregulation in conjunction with hormone replacement therapy on endometrial preparation in patients for frozen-thawed embryo transfer. *Frontiers in Medicine*. 2024; 11: 1412126. <https://doi.org/10.3389/fmed.2024.1412126>
- [5] Clemenza S, Vannuccini S, Ruotolo A, Capezzuoli T, Petraglia F. Advances in targeting estrogen synthesis and receptors in patients with endometriosis. *Expert Opinion on Investigational Drugs*. 2022; 31: 1227–1238. <https://doi.org/10.1080/13543784.2022.2152325>
- [6] Agarwal SK, Foster WG. Reduction in Endometrioma Size with Three Months of Aromatase Inhibition and Progestin Add-Back. *BioMed Research International*. 2015; 2015: 878517. <https://doi.org/10.1155/2015/878517>
- [7] Timmerman D, Valentin L, Bourne TH, Collins WP, Verrelst H, Vergote I, et al. Terms, definitions and measurements to describe the sonographic features of adnexal tumors: a consensus opinion from the International Ovarian Tumor Analysis (IOTA) Group. *Ultrasound in Obstetrics & Gynecology: the Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*. 2000; 16: 500–505. <https://doi.org/10.1046/j.1469-0705.2000.00287.x>
- [8] La Marca A, Sunkara SK. Individualization of controlled ovarian stimulation in IVF using ovarian reserve markers: from theory to practice. *Human Reproduction Update*. 2014; 20: 124–140. <https://doi.org/10.1093/humupd/dmt037>
- [9] Chen ZJ, Liu JY, Huang HF, Qiao J, Zhou CQ, Huang GN, et al. Guidelines for the diagnosis and treatment of infertility. *Chinese Journal of Obstetrics and Gynecology*. 2019; 54: 505–511. <https://doi.org/10.3760/cma.j.issn.0529-567x.2019.08.001> (In Chinese)
- [10] Lang JH, Leng JH, Yang Q, Li R, Hua KQ, Zhang GN, et al. Chinese Expert Consensus on GnRH-a in Obstetrics and Gynecology. *Zhonghua Fu Chan Ke Za Zhi*. 2025; 60: 913–925. <https://doi.org/10.3760/cma.j.cn112141-20250529-00244> (In Chinese)
- [11] Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting.

- Human Reproduction. 2011; 26: 1270–1283. <https://doi.org/10.1093/humrep/der037>
- [12] Sun Q, Cao Y, Gu J, Xu Y. The application of progesterone-primed ovarian stimulation protocol in patients with ovarian endometriosis combined with diminished ovarian reserve. *BMC Women's Health*. 2025; 25: 70. <https://doi.org/10.1186/s12905-025-03601-w>
- [13] Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K, et al. The International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) Revised Glossary on ART Terminology, 2009. *Human Reproduction*. 2009; 24: 2683–2687. <https://doi.org/10.1093/humrep/dep343>
- [14] Penzer AJ, Schweikart SJ. Using Policy and Law to Help Reduce Endometriosis Diagnostic Delay. *AMA Journal of Ethics*. 2025; 27: E104–109. <https://doi.org/10.1001/amajethics.2025.104>
- [15] Hashemi M, Mohseni H, Ghahiri A, Rouholamin S. Laparoscopic and Hysteroscopic Findings in A Selected Group of Women with Unexplained Infertility: A Cross-Sectional Study. *International Journal of Fertility & Sterility*. 2024; 18: 339–343. <https://doi.org/10.22074/ijfs.2023.2005476.1484>
- [16] Latif S, Khanjani S, Saridogan E. Endometriosis and In Vitro Fertilization. *Medicina*. 2024; 60: 1358. <https://doi.org/10.3390/medicina60081358>
- [17] Oral E, Arici A, Olive DL, Huszar G. Peritoneal fluid from women with moderate or severe endometriosis inhibits sperm motility: the role of seminal fluid components. *Fertility and Sterility*. 1996; 66: 787–792. [https://doi.org/10.1016/s0015-0282\(16\)58637-3](https://doi.org/10.1016/s0015-0282(16)58637-3)
- [18] Liu Y, Luo L, Zhao H. Changes of tumor necrosis factor alpha and interleukin-6 levels in peritoneal fluid of patients with endometriosis and their effects on human sperm motility and mouse embryo development. *Zhonghua Fu Chan Ke Za Zhi*. 2000; 35: 332–334. (In Chinese)
- [19] Paffoni A, Bolis V, Ferrari S, Benaglia L, Vercellini P, Somigliana E. The Gametotoxic Effects of the Endometrioma Content: Insights From a Parthenogenetic Human Model. *Reproductive Sciences*. 2019; 26: 573–579. <https://doi.org/10.1177/1933719118777637>
- [20] Veth VB, van de Kar MM, Duffy JM, van Wely M, Mijatovic V, Maas JW. Gonadotropin-releasing hormone analogues for endometriosis. *The Cochrane Database of Systematic Reviews*. 2023; 6: CD014788. <https://doi.org/10.1002/14651858.CD014788.pub2>
- [21] Becker CM, Bokor A, Heikinheimo O, Horne A, Jansen F, Kiesel L, et al. ESHRE guideline: endometriosis. *Human Reproduction Open*. 2022; 2022: hoac009. <https://doi.org/10.1093/hropen/hoac009>
- [22] Söritsa D, Saare M, Laisk-Podar T, Peters M, Söritsa A, Matt K, et al. Pregnancy rate in endometriosis patients according to the severity of the disease after using a combined approach of laparoscopy, GnRH agonist treatment and in vitro fertilization. *Gynecologic and Obstetric Investigation*. 2015; 79: 34–39. <https://doi.org/10.1159/000365329>
- [23] Filindris T, Papakonstantinou E, Keramida M, Panteris E, Kalogeropoulos S, Georgopoulos N, et al. The effect of GnRH-a on the angiogenesis of endometriosis. *Hormones*. 2024; 23: 509–515. <https://doi.org/10.1007/s42000-024-00559-6>
- [24] Tamura H, Takasaki A, Nakamura Y, Numa F, Sugino N. A pilot study to search possible mechanisms of ultralong gonadotropin-releasing hormone agonist therapy in IVF-ET patients with endometriosis. *Journal of Ovarian Research*. 2014; 7: 100. <https://doi.org/10.1186/s13048-014-0100-8>
- [25] 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. <https://doi.org/10.1016/j.repbio.2022.100608>
- [26] Panagodimou EK, Kalogeropoulos S, Adonakis G, Kaponis A. Does Gonadotropin-Releasing Hormone Agonist Administration Before Assisted Reproduction Techniques Improve Pregnancy Rates in Women With Endometriosis? *Obstetrical & Gynecological Survey*. 2024; 79: 421–428. <https://doi.org/10.1097/OGX.0000000000001283>
- [27] Suzuki R, Horage-Okutsu Y, Kawahara T, Nakamura K, Shiraishi E, Iwahata H, et al. The effect of aromatase inhibitor on controlled ovarian stimulation for oocyte cryopreservation in adolescent and young cancer patients. *The Journal of Obstetrics and Gynaecology Research*. 2023; 49: 973–979. <https://doi.org/10.1111/jog.15535>
- [28] Shapira M, Orvieto R, Lebovitz O, Nahum R, Aizer A, Segev-Zahav A, et al. Does daily co administration of gonadotropins and letrozole during the ovarian stimulation improve IVF outcome for poor and sub optimal responders? *Journal of Ovarian Research*. 2020; 13: 66. <https://doi.org/10.1186/s13048-020-00666-z>
- [29] Elgindy EA, Abdelghany AA, Sibai AbdAlsalam H, Mostafa MI. The novel incorporation of aromatase inhibitor in hormonal replacement therapy cycles: a randomized controlled trial. *Reproductive Biomedicine Online*. 2022; 44: 641–649. <https://doi.org/10.1016/j.rbmo.2021.10.025>
- [30] Zhang J, Wang L, Li C, Zhang H, Li R, Li M. Letrozole promotes the expression of integrin $\alpha v \beta 3$ and HOXA10 in endometrium of endometriosis. *Systems Biology in Reproductive Medicine*. 2022; 68: 121–128. <https://doi.org/10.1080/19396368.2021.2013577>
- [31] Steiner N, Shrem G, Tannus S, Dahan SY, Balayla J, Volodarsky-Perel A, et al. Effect of GnRH agonist and letrozole treatment in women with recurrent implantation failure. *Fertility and Sterility*. 2019; 112: 98–104. <https://doi.org/10.1016/j.fertnstert.2019.03.021>
- [32] Cozzolino M, Pellicer N, Galliano D, Pellicer A. Pituitary suppression with GnRH agonists before ART may be insufficient to treat women with severe adenomyosis. *Reproductive Biomedicine Online*. 2023; 46: 150–155. <https://doi.org/10.1016/j.rbmo.2022.09.023>
- [33] Piedimonte S, Volodarsky-Perel A, Tannus S, Tan SL, Dahan MH. Pretreatment with a gonadotropin-releasing hormone agonist and an aromatase inhibitor may improve outcomes in in vitro fertilization cycles of women with stage I-II endometriosis. *F&S Science*. 2020; 1: 98–103. <https://doi.org/10.1016/j.xfss.2020.06.005>
- [34] Cantor A, Tannus S, Son WY, Tan SL, Dahan MH. A comparison of two months pretreatment with GnRH agonists with or without an aromatase inhibitor in women with ultrasound-diagnosed ovarian endometriomas undergoing IVF. *Reproductive Biomedicine Online*. 2019; 38: 520–527. <https://doi.org/10.1016/j.rbmo.2018.12.028>
- [35] Yoshihara K. Pathogenesis of endometrium-related diseases based on genomic alterations in normal uterine endometrium. *The Journal of Obstetrics and Gynaecology Research*. 2023; 49: 2023–2030. <https://doi.org/10.1111/jog.15680>
- [36] Pritts EA. Letrozole for ovulation induction and controlled ovarian hyperstimulation. *Current Opinion in Obstetrics & Gynecology*. 2010; 22: 289–294. <https://doi.org/10.1097/GCO.0b013e32833bee6f>
- [37] Tatsumi T, Jwa SC, Kuwahara A, Irahara M, Kubota T, Saito H. No increased risk of major congenital anomalies or adverse pregnancy or neonatal outcomes following letrozole use in assisted reproductive technology. *Human Reproduction*. 2017; 32: 125–132. <https://doi.org/10.1093/humrep/dew280>