Drug Design, Development and Therapy

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ORIGINAL RESEARCH

The Efficacy of Letrozole Co-Treatment in an Antagonist Protocol for Women with Polycystic Ovary Syndrome Undergoing IVF: A Retrospective Study

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Objective: Our objective was to investigate the efficacy of letrozole co-treatment in an antagonist protocol for infertile women with polycystic ovary syndrome (PCOS).

Patients and Methods: This retrospective cohort study included infertile women with PCOS undergoing IVF/ICSI with and without letrozole co-treatment in an antagonist protocol from 2007–2021 at Shanghai Ninth People's Hospital (Shanghai, China). A total of 1559 participants were enrolled, with 1227 women in the antagonist group and 332 women in the letrozole co-treatment group. Propensity score-based patient-matching model was conducted to balance covariates between the groups. The primary outcome was the number of retrieved oocytes, with secondary outcomes including endocrine parameters, ovarian stimulation outcomes, pregnancy outcomes, and obstetrical and neonatal complications.

Results: Letrozole co-treatment induced significant changes in hormonal regulation, increased the percentage of large follicles, and resulted in fewer retrieved oocytes ($P < 0.05$). However, there was no negative impact on the number of usable embryos or good-quality embryos ($P > 0.05$). The live birth rates following fresh embryo transfer were comparable between the letrozole and control groups (single embryo transfer: 28.9% vs 29.7%, $P > 0.05$; double embryo transfer: 37.3% vs 45.6%, $P > 0.05$). Additionally, there were no significant differences between the two groups in the live birth rate per patient after frozen embryo transfer and the cumulative live birth rate ($P > 0.05$). No significant differences in obstetrical and neonatal complications were observed between the groups ($P > 0.05$).

Conclusion: The addition of letrozole to the antagonist protocol for women with PCOS undergoing IVF induces a higher percentage of large follicles during oocyte retrieval, while reducing the overall number of retrieved oocytes. Moreover, the use of letrozole demonstrates comparable clinical outcomes following embryo transfers. These findings highlight the potential application of letrozole in an antagonist protocol for women with PCOS.

Keywords: letrozole, antagonist protocol, polycystic ovary syndrome, follicle, in vitro fertilization

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting approximately 5–10% of women of reproductive age worldwide.[1] Characterized by hyperandrogenism, ovulatory dysfunction, and the presence of polycystic ovaries,[2] PCOS often leads to infertility, prompting the use of assisted reproductive technologies such as in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) for achieving pregnancy.[3] However, women with PCOS undergoing IVF/ICSI encounter various challenges, including ovarian hyperstimulation syndrome (OHSS), poor oocyte quality, and reduced pregnancy rates.[3]

Received: 19 February 2024
Accepted: 27 June 2024
Published: 8 July 2024

One potential approach to address these challenges is through frozen embryo transfer (FET), which has shown promise in improving the live birth rate and minimizing the risks of OHSS and pregnancy complications in women with PCOS.[4] Despite the advantages of FET, it is noteworthy that it may be associated with a higher incidence of preeclampsia,[5] making fresh embryo transfer the preferred choice in IVF procedures for patients striving to achieve clinical pregnancy and live birth in the shortest possible time.

In the quest to overcome the challenges faced by women with PCOS during IVF/ICSI, researchers have explored alternative approaches that facilitate embryo transfer (ET) in a more natural hormonal and uterine environment. Letrozole, an aromatase inhibitor, emerges as a promising ovulation induction agent in women with PCOS.[6] By inhibiting the conversion of androgens to estrogens in ovarian granulosa cells, letrozole effectively reduces both serum and follicular estrogen levels.[7] Integrating letrozole into a gonadotropin-releasing hormone (GnRH) antagonist protocol has gained acceptance as a treatment option for oocyte retrieval in women with poor ovarian response, aiming to enhance follicular response and improve oocyte quality.[8] Recent research has highlighted the potential benefits of letrozole co-treatment extending into the luteal phase, mitigating the detrimental effects of accumulating estradiol concentrations on both oocyte quality and endometrial receptivity.[9,10] Additionally, letrozole administration may lower intraovarian and serum estrogen levels, potentially mitigating the risk of OHSS in normal women undergoing IVF.[11] Previous studies have mainly focused on the clinical effects of letrozole co-administration in normal and poor ovarian responders,[12] raising uncertainties regarding the potential benefits of letrozole in IVF antagonist protocol for women with PCOS.

Therefore, this retrospective study was conducted to evaluate the efficacy of letrozole co-administration in an antagonist protocol on IVF/ICSI outcomes in infertile women with PCOS, aiming to address the existing research gap and contribute to the body of knowledge in this area. The utilization of a propensity score-based patient-matching (PSM) method allowed for the creation of matched groups with similar baseline characteristics, ensuring a stronger causal relationship between the treatment and outcomes.[13] By comprehensively investigating the role of letrozole in IVF treatment for women with PCOS, this study aimed to provide valuable insights for improving reproductive outcomes in this patient population.

Patients and Methods

Study Design and Participants

The study was a retrospective cohort study conducted in Shanghai Ninth People's Hospital of Shanghai Jiao Tong University School of Medicine (Shanghai, China) from 2007 to 2021. Study participants consisted of women diagnosed with PCOS who underwent IVF/ICSI treatment with or without co-administration of letrozole in an antagonist protocol at Shanghai Ninth People's Hospital between 2007 and 2021 (Supplementary Figure S1). The diagnosis of PCOS was based on the Rotterdam consensus criteria, which required the presence of at least two out of three criteria: oligo- or anovulation, clinical or biochemical signs of hyperandrogenism, and polycystic ovaries, while ruling out other potential causes.[14] To maintain homogeneity within the sample as well as minimize confounding effects and potential risks associated with advanced maternal age and severe medical condition, women were excluded if they were over the age of 40, had a history of failed IVF/ICSI attempts, recurrent spontaneous abortion, chromosomal abnormalities, severe conditions such as liver, kidney, and immune-related diseases, or incomplete data.

IVF/ICSI-ET Procedures

Participants received gonadotropin at a daily dose of 75 to 300 IU from cycle day 2–3, with or without co-treatment of letrozole (2.5 mg/day or 5 mg/day for 5 consecutive days) until the trigger day.[15] GnRH antagonist was administered when a dominant follicle reached a mean diameter of 13–14 mm or when blood luteinizing hormone (LH) levels showed a significant upward trend, continuing until and including the trigger day.[16] Final oocyte maturation was induced by administering triptorelin 0.1 mg and human chorionic gonadotropin (hCG) 2000 IU when at least two leading follicles were ≥ 18 mm or more. Oocyte retrieval was performed 34–36 hours after hCG injection and the oocytes were fertilized through IVF, ICSI, or IVF+ICSI determined by sperm quality. The quality of embryos was evaluated based on the

Istanbul Consensus.[17] A Fresh ET was performed according to clinical practice, with surplus embryos vitrified for subsequent FET. All patients received luteal support post-ET.

Data Collection and Outcome Measures

Clinical data were extracted from electronic medical records. A blinded process was implemented throughout the stages of data collection, input, and verification within the database, as well as during data download, analysis, and result validation.

The primary outcome of the study was the number of oocytes retrieved. Secondary outcomes included: (1) follicular phase endocrine parameters, (2) ovarian stimulation outcomes, (3) pregnancy rate and live birth rate, (4) obstetrical and neonatal outcomes. Hormonal levels of oestradiol (E2), progesterone (P), follicle-stimulating hormone (FSH), and LH were collected at the start of stimulation and on the day of ovulation trigger. The cycle cancellation rate was estimated by assessing the number of patients who did not have viable embryos after oocyte retrieval. Clinical pregnancy was determined by transvaginal ultrasound showing at least one intrauterine gestational sac 35 days after ET. Live birth was defined as the delivery of at least one live-born infant, irrespective of gestational duration. Obstetrical outcomes, including twin pregnancy, hypertensive disorders in pregnancy, gestational diabetes, placental previa, preterm premature rupture of the membranes, and cesarean delivery, were evaluated for live births resulting from fresh ET cycles. Neonatal outcomes, including neonatal death, birth defects, a composite of neonatal morbidity (neonatal jaundice, pneumonia, necrotizing enterocolitis, respiratory distress syndrome, sepsis, hypoxic ischemic encephalopathy), gestational age, and birth weight, were also collected. Adverse events were recorded at clinic visits until a negative pregnancy test or through a telephone follow-up until fetal birth.

Statistical Analyses

The Shapiro–Wilk test was used to assess the distributions of continuous variables. Normally distributed variables were presented as means (standard deviations), non-normally distributed variables as medians (interquartile ranges), and categorical variables as numbers (percentages). Baseline characteristics were compared between groups using Student's t-tests for normally distributed variables and with Chi-square test for categorical variables. Differences in endocrine measurements were compared using Student's t-tests on log-transformed concentrations. To balance the covariates between the two groups, a PSM model was utilized using R package MatchIt. The selection of the minimum adjusted set of covariates in the model was based on their potential confounding relationships with both the exposure and outcome variables, in addition to considerations of clinical judgment and existing literature. The following pretreatment characteristics were included as covariates: age, pregestational body mass index (BMI), infertility cause, infertility duration, gravidity, parity, antral follicle count (AFC), and fertilization method. Cases with and without letrozole co-treatment were matched based on estimated propensity scores obtained from a binary logistic regression analysis. Patients were matched (1:1) using the nearest neighbor method (distance = "glm") without replacement and a caliper of 0.05. Standardized mean difference (SMD) was calculated using R package cobalt; covariates were considered well-balanced when SMD was below 5% (Supplementary Figure S2). The effect of letrozole co-treatment was then estimated in the matched cohort.

Sample size calculation and power analysis were performed using G*Power v 3.1. All statistical analyses were conducted using R v 4.2.2. A two-sided *P*-value less than 0.05 was considered statistically significant.

Ethical Approval

The study followed the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of Shanghai Ninth People's Hospital of Shanghai Jiao Tong University School of Medicine (Shanghai, China) (No. SH9H-2021-T467-2). All retrieved data underwent de-identification procedures to ensure the protection of patient confidentiality. Due to the retrospective nature of all analyses and the deidentification of data, the requirement for informed consent was waived by the Institutional Review Board of Shanghai Ninth People's Hospital.

Results

Inclusion and Baseline Data

A total of 1559 participants diagnosed with PCOS and undergoing IVF/ICSI treatment were included in the study. The participants were divided into two groups: 1227 women in the GnRH antagonist group and 332 women in the letrozole co-treatment group. The demographic characteristics of the participants before and after PSM are summarized in Table 1. After PSM, the baseline characteristics of the two groups, including maternal age at oocyte retrieval, gravidity, parity, infertility cause, infertility duration, BMI, AFC, and fertilization method, were found to be comparable ($P > 0.05$).

IVF Cycle Endocrinology

The endocrinology of the IVF cycles was analyzed, and the hormone profile after PSM is presented in Figure 1. The baseline levels of E2, P, FSH, and LH did not differ significantly between the two groups ($P > 0.05$). However, the letrozole co-treatment group exhibited significantly lower E2 concentrations on trigger day compared to the antagonist group (914 [456, 1756] vs 3874 [2616, 5000], $P < 0.001$). Additionally, the letrozole co-treatment group had significantly lower FSH concentrations (10.7 [8.5, 13.4] vs 12.4 [10.5, 14.7], $P < 0.001$) and higher LH concentrations (3.09 [1.82, 5.59] vs 1.79 [1.07, 3.00], $P < 0.001$) on trigger day compared to the antagonist group. No significant between-group differences were found in P levels ($P > 0.05$). During controlled ovarian stimulation, patients co-treated with letrozole received a lower dose of gonadotropins (1384 ± 839 vs 1929 ± 798, $P < 0.001$) and had a shorter stimulation duration (8.55 ± 2.82 vs 9.40 ± 2.44, $P < 0.001$) than the control group.

Table 1 Study Participant Characteristics Before and After PSM

	Before PSM			After PSM		
	Antagonist Group (n = 1227)	**Letrozole Group (n = 332)**	***P* value**	**Antagonist Group (n = 328)**	**Letrozole Group (n = 328)**	***P* value**
Maternal age at oocyte retrieval (years), mean (SD)	30.5 (3.50)	30.2 (3.45)	0.103	30.0 (3.39)	30.2 (3.45)	0.565
BMI (kg/m^2), n (%)			0.157			0.651
<18.5	99 (8.07%)	30 (9.04%)		38 (11.6%)	29 (8.84%)	
18.5–24	675 (55.0%)	198 (59.6%)		194 (59.1%)	195 (59.5%)	
24–28	308 (25.1%)	69 (20.8%)		68 (20.7%)	69 (21.0%)	
>28	134 (10.9%)	29 (8.73%)		25 (7.62%)	29 (8.84%)	
Duration of infertility (years), mean (SD)	3.56 (2.35)	3.60 (2.09)	0.813	3.52 (2.26)	3.59 (2.10)	0.668
Infertility cause, n (%)			0.052			0.910
Tubal	538 (43.8%)	171 (51.5%)		155 (47.3%)	169 (51.5%)	
Ovulatory	203 (16.5%)	61 (18.4%)		62 (18.9%)	59 (18.0%)	
Uterine	26 (2.12%)	4 (1.20%)		5 (1.52%)	4 (1.22%)	
Male	330 (26.9%)	70 (21.1%)		74 (22.6%)	70 (21.3%)	
Unexplained	74 (6.03%)	17 (5.12%)		21 (6.40%)	17 (5.18%)	
Gravidity, n (%)			0.502			0.708
0	836 (68.1%)	215 (64.8%)		220 (67.1%)	213 (64.9%)	
1	237 (19.3%)	70 (21.1%)		69 (21.0%)	69 (21.0%)	
≥2	154 (12.6%)	47 (14.2%)		39 (11.9%)	46 (14.0%)	
Primiparous, n (%)	1125 (91.7%)	310 (93.4%)	0.372	312 (95.1%)	306 (93.3%)	0.403
AFC, mean (SD)	20.0 (7.18)	21.2 (8.33)	0.019	21.0 (7.57)	20.9 (7.90)	0.896
Fertilization method, n (%)			<0.001			0.904
IVF	580 (47.3%)	199 (59.9%)		187 (57.0%)	195 (59.5%)	
ICSI	359 (29.3%)	93 (28.0%)		98 (29.9%)	93 (28.4%)	
IVF+ICSI	280 (22.8%)	37 (11.1%)		39 (11.9%)	37 (11.3%)	

Abbreviations: AFC, antral follicle count; BMI, body mass index; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; PSM, propensity score-based patient-matching; SD, standard deviation.

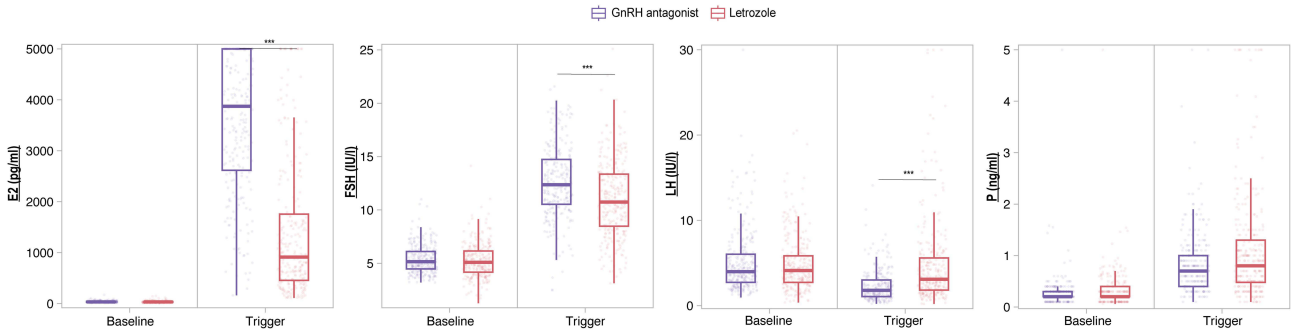

Figure 1 Hormone profiles in women with PCOS undergoing IVF with and without letrozole co-treatment. Hormone levels were measured at the start of ovarian stimulation and on the day of the ovulation trigger. The data are presented using box plots, which visually display the median, interquartile range (representing the middle 50% of the values), and the range (excluding any outliers). Statistical significance is indicated as "***" for $P < 0.001$.
Abbreviations: E2, oestradiol; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; P, progesterone.

Follicle Development and Embryonic Parameters

The follicle recruitment and embryonic parameters were assessed, and the results are depicted in Figure 2. After PSM, there was no significant difference in AFC between the groups (20.9 ± 7.90 vs 21.0 ± 7.57, $P = 0.896$). However, the total number of follicles was found to be decreased in the letrozole group compared to the antagonist group (15.2 ± 8.71 vs 20.9 ± 8.44, $P < 0.001$; Figure 2A). Interestingly, there was a significant difference in the percentage of large follicles (>16mm) at oocyte retrieval, with a higher proportion observed in the letrozole group (48% vs 38%, $P < 0.001$; Figure 2B). Letrozole administration was associated with a reduced number of retrieved oocytes (11.9 ± 9.14 vs 15.5 ± 8.05, $P < 0.001$; Figure 3), resulting in a subsequent decrease in the number of matured and fertilized oocytes, as well as cleaved embryos (Figure 3). However, this difference did not impact the number of usable embryos (5.11 ± 3.87 vs 5.36 ± 3.54, $P = 0.388$) or the number of good-quality embryos (4.48 ± 4.02 vs 4.69 ± 3.91, $P = 0.485$) (Figure 3). While the two groups exhibited comparable oocyte retrieval rate (74% vs 74%, $P = 0.874$) and maturation rate (78% vs 81%, $P = 0.073$), the letrozole group demonstrated a higher fertilization rate (82% vs 77%, $P = 0.001$) and usable embryo rate (74% vs 56%, $P < 0.001$). There was no significant difference in the cycle cancellation rate for nonviable embryos between the two groups (5.18% vs 8.54%, $P = 0.122$; Table 2).

Pregnancy Outcomes

Letrozole co-treatment resulted in a higher rate of fresh ET compared to the antagonist group (58.5% vs 44.5%, $P < 0.001$). The pregnancy outcomes following fresh ET were stratified by the number of embryos transferred, and the results are

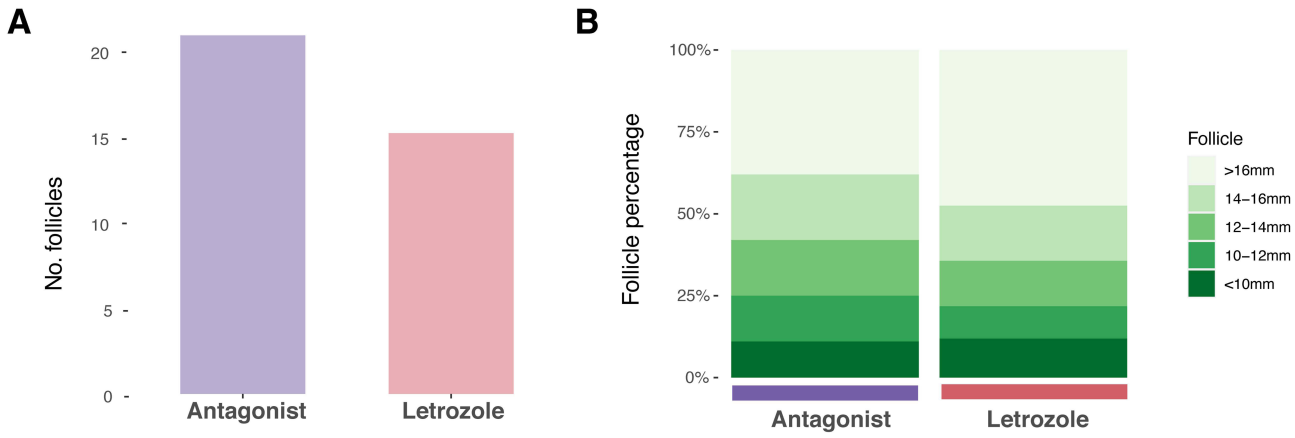

Figure 2 Follicle recruitment in women with PCOS undergoing IVF with and without letrozole co-treatment. (**A**) Comparison of the number of follicles. The numbers are depicted using purple bars (GnRH antagonist group) and red bars (letrozole co-treatment group). (**B**) The percentage of follicles categorized by size. Data are represented by a color gradient from light green to dark green, corresponding to the follicle size (>16mm, 14–16mm, 12–14mm, 10–12mm, and <10mm), respectively.

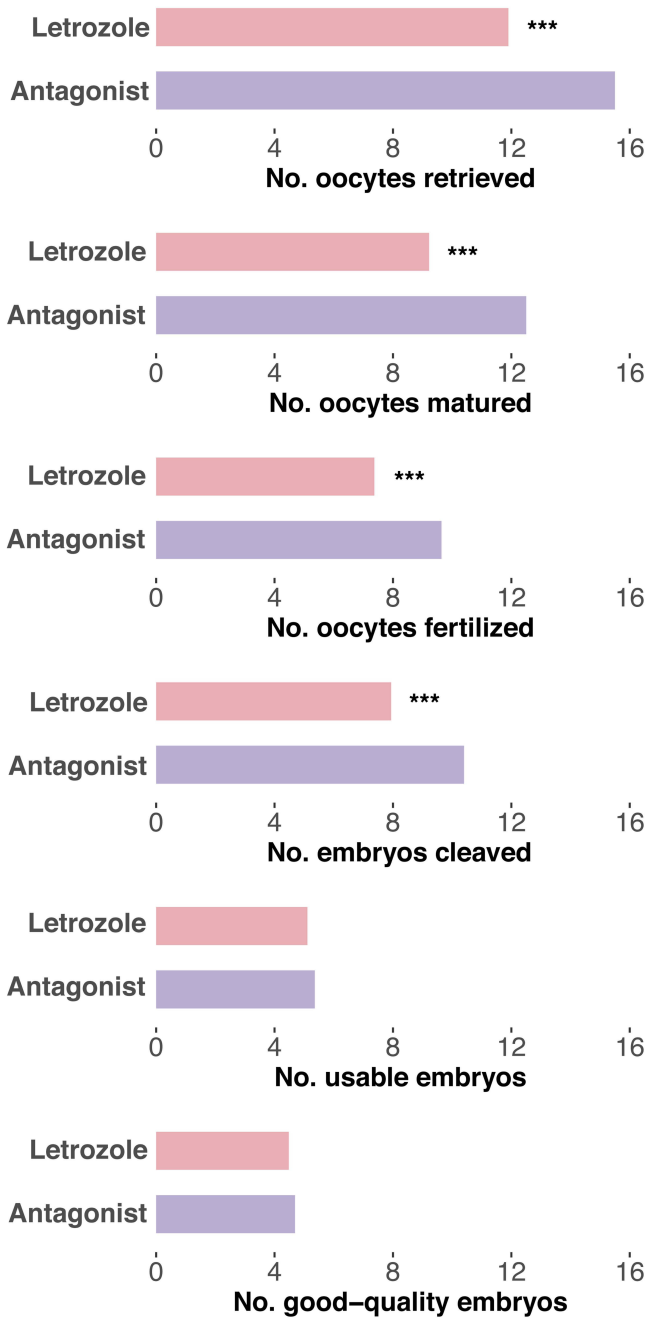

Figure 3 Embryonic parameters in women with PCOS undergoing IVF with and without letrozole co-treatment. Comparison of the numbers of retrieved, matured and fertilized oocytes, as well as the numbers of cleaved, usable, and good-quality embryos. The numbers are depicted using purple bars (GnRH antagonist group) and red bars (letrozole co-treatment group). Statistical significance is indicated as "***" for $P < 0.001$.

summarized in Table 2. The endometrial thickness on the day of transfer was similar between the two groups ($P > 0.05$; Table 2). There was no significant difference in the stage of embryos transferred ($P > 0.05$; Table 2). The live birth rates after fresh ET were comparable between the letrozole and control groups (single embryo transfer: 28.9% vs 29.7%, $P = 1.000$; double embryo transfer: 37.3% vs 45.6%, $P = 1.000$).

The pregnancy outcomes following FET are presented in Table 2. The letrozole group tended to transfer a higher number of embryos per cycle, resulting in a higher live birth rate per FET cycle ($P < 0.05$). As the number of embryos transferred per patient was similar between the groups, the live birth rate per patient was comparable, with no statistically

Table 2 IVF Pregnancy Outcomes After PSM

	Antagonist Group	Letrozole Group	*P* value
Cycle cancellation rate (%)	28 (8.54%)	17 (5.18%)	0.122
Fresh cycle outcomes			
Patients/cycles	n = 146	n = 192	
Endometrial thickness at transfer day (mm), mean (SD)	12.0 (2.16)	12.5 (2.92)	0.376
Number of embryos transferred, mean (SD)	1.55 (0.50)	1.77 (0.44)	<0.001
Stage of embryos transferred, n (%)			1.000
Cleavage-stage embryo	143 (97.9%)	188 (97.9%)	
Blastocyst	3 (2.05%)	3 (1.56%)	
Clinical pregnancy, n (%)	88 (38.1%)	89 (38.5%)	1.000
Live birth, n (%)	56 (38.4%)	66 (34.4%)	0.522
Single embryo transfer			
Clinical pregnancy, n (%)	24 (37.5%)	16 (35.6%)	0.996
Live birth, n (%)	19 (29.7%)	13 (28.9%)	1.000
Double embryo transfer			
Clinical pregnancy, n (%)	39 (49.4%)	69 (48.6%)	1.000
Live birth, n (%)	36 (45.6%)	53 (37.3%)	0.292
Frozen-thawed cycle outcomes			
Patients	n = 212	n = 196	
Average number of embryos transferred per patient, mean (SD)	2.74 (1.70)	2.66 (1.63)	0.660
Clinical pregnancy rate per patient, n (%)	151 (71.2%)	131 (66.8%)	0.394
Live birth rate per patient, n (%)	115 (54.2%)	115 (58.7%)	0.423
Cycles	n = 348	n = 278	
Average number of embryos transferred per cycle, mean (SD)	1.65 (0.43)	1.85 (0.48)	<0.001
Clinical pregnancy rate per ET, n (%)	151 (43.4%)	131 (47.1%)	0.395
Live birth rate per ET, n (%)	115 (33.0%)	115 (41.4%)	0.039
Cumulative outcomes			
Cumulative live birth rate, n (%)	168 (56.0%)	177 (56.9%)	0.884

Abbreviation: SD, standard deviation.

significant difference observed (58.7% vs 54.2%, $P = 0.423$). The cumulative live birth rate did not differ significantly between the letrozole and control groups (56.9% vs 56.0%, $P = 0.884$).

Obstetrical and Neonatal Outcomes

Regarding obstetrical and neonatal outcomes (Table 3), no significant associations were found between letrozole co-treatment and twin pregnancy, hypertensive disorders in pregnancy, gestational diabetes, placental previa, preterm

Table 3 Associations Between Letrozole Co-Treatment and Obstetrical/Neonatal Complications After PSM

Outcomes	Antagonist Group (n = 56)	Letrozole Group (n = 66)	*P* value
Maternal			
Twin pregnancy	8 (14.3%)	9 (13.6%)	1.000
Hypertensive disorders in pregnancy	4 (7.14%)	4 (6.06%)	1.000
Gestational diabetes	3 (5.36%)	4 (6.06%)	1.000
Placenta previa	0 (0.00%)	1 (1.52%)	1.000
Preterm premature rupture of the membranes	2 (3.57%)	3 (4.55%)	1.000
Cesarean delivery	37 (66.1%)	48 (72.7%)	0.549

(*Continued*)

Table 3 (Continued).

Outcomes	Antagonist Group (n = 56)	Letrozole Group (n = 66)	*P* value
Neonatal			
Gestational age, wk	38.1 (2.15)	38.1 (2.09)	0.941
Preterm birth, <37 weeks	8 (14.3%)	9 (13.6%)	1.000
Very preterm birth, <32 weeks	2 (3.57%)	2 (3.03%)	1.000
Birth weight, g	3138 (528)	3164 (525)	0.791
Low birth weight, <2500 g	8 (14.3%)	7 (10.6%)	0.734
Small for gestational age	8 (14.3%)	4 (6.06%)	0.224
Macrosomia, >4000 g	3 (5.36%)	4 (6.06%)	1.000
Large for gestational age	9 (16.1%)	10 (15.2%)	1.000
Neonatal death	1 (1.79%)	0 (0.00%)	0.459
Birth defect	1 (1.79%)	1 (1.52%)	1.000
Morbidity	3 (5.36%)	1 (1.52%)	0.332
Neonatal jaundice	1 (1.79%)	0 (0.00%)	0.459
Pneumonia	0 (0.00%)	0 (0.00%)	NA
Necrotizing enterocolitis	0 (0.00%)	0 (0.00%)	NA
Respiratory distress syndrome	2 (3.57%)	1 (1.52%)	0.593
Sepsis	0 (0.00%)	0 (0.00%)	NA
Hypoxic ischemic encephalopathy	0 (0.00%)	0 (0.00%)	NA

Abbreviation: NA, not applicable.

premature rupture of the membranes, or cesarean delivery ($P > 0.05$). Furthermore, there were no significant differences between the two groups in terms of gestational age (preterm birth, very preterm birth), birth weight (low birth weight, macrosomia, large for gestational age, small for gestational age), neonatal death, birth defects, or a composite of neonatal morbidity (including neonatal jaundice, necrotizing enterocolitis, pneumonia, respiratory distress syndrome, sepsis, and hypoxic ischemic encephalopathy) ($P > 0.05$).

Discussion

The present study provides an extensive exploration into the endocrine effects and clinical outcomes of letrozole co-treatment in women with PCOS undergoing IVF treatment. Our findings reveal that letrozole co-treatment induces significant changes in hormonal regulation, leading to an increase in the percentage of large follicles at oocyte retrieval while decreasing the number of retrieved oocytes. Letrozole co-treatment resulted in an elevated fertilization rate and usable embryo rate as well as a higher rate of fresh ET. Despite these changes, we did not observe any statistically significant differences in pregnancy outcomes after ETs.

Hormonal Profiles and Follicular Development

Our analysis of endocrine parameters demonstrated that letrozole effectively suppressed E2 levels on the trigger day in women with PCOS, which is consistent with previous studies investigating letrozole administration.[18–20] Moreover, we found that letrozole had an upregulating effect on LH levels during the follicular phase, aligning with the results of two randomized controlled trials.[9,21] Interestingly, our study revealed a significantly lower FSH concentration in the letrozole co-treatment group, contradicting the findings of other studies that reported increased FSH levels following letrozole administration.[9,21] Similar to previous studies, we also observed lower exogenous gonadotropin consumption and shorter stimulation time in women co-treated with letrozole, supporting the notion of increased follicular sensitivity to exogenous FSH.[11,19,22] The observed increase in the percentage of large follicles following letrozole co-treatment is likely a consequence of the altered endocrinology. The finding is consistent with previous studies conducted on both

poor- and normo-responders, which have reported an increased number of large follicles in women co-treated with letrozole.[20,23]

Potential Mechanisms

Letrozole, functioning as an aromatase inhibitor, inhibits the synthesis of estrogen and reduces negative estrogenic feedback to the hypothalamic/pituitary axis, resulting in the increased secretion of endogenous gonadotrophins.[24,25] Moreover, the elevated levels of LH can facilitate the selection of dominant follicles and promote the growth of the dominant follicle after the initiation of diameter deviation.[26,27] Another possible explanation for the increased follicle diameter in the letrozole group may be attributed to the effects of androgens. Previous studies have indicated that the letrozole group exhibited higher levels of serum testosterone and androstenedione starting from stimulation day 5 onwards.[9] The accumulation of androgen concentrations could be a result of aromatase inhibition, preventing the conversion of androgens into estrogens. Another plausible mechanism could be the increased activity of the cytochrome p450 CYP17A enzyme in the theca cells, induced by letrozole cotreatment. The elevated levels of LH and inhibin B, caused by letrozole, act synergistically to enhance androgen production by the theca cells.[28] Androgens are known to augment follicular sensitivity to FSH and promote early follicular growth by overexpression and sensitization of FSH receptors.[29] Furthermore, androgens have been found to attenuate follicular atresia.[30]

Oocyte Retrieval

Conflicting results have been reported regarding the impact of letrozole co-treatment on the number of aspirated oocytes. A meta-analysis incorporating 26 studies with 3091 women has displayed that letrozole co-treatment has no effect on the number of aspirated oocytes.[31] We observed a significant decrease in the number of retrieved oocytes during letrozole co-treatment (11.9 ± 9.14 vs 15.5 ± 8.05, $P < 0.001$). The optimal number of retrieved oocytes in order to maximize the chances of a successful pregnancy and live birth while minimizing complications has been a topic of interest in the management of PCOS patients. Previous studies have suggested that there may be a threshold beyond which additional oocytes do not contribute to higher success rates. A recent study has summarized that the optimal number of retrieved oocytes differs depending on whether fresh ET or FET is performed.[32] For fresh ET, the live birth rate increases by 12% with each additional oocyte up to a threshold of 11 oocytes, beyond which the rate plateaus. In contrast, for FET, PCOS patients achieve the highest live birth rate when 25 oocytes are retrieved, and the rate stabilizes thereafter. The cumulative live birth rate plateaus at 15 oocytes. It is important to note that retrieving more than 15 oocytes in a fresh ET cycle increases the risk of OHSS, but does not improve the live birth rate.[33] A study has found that PCOS patients with 10–15 retrieved oocytes had a cumulative live birth rate of 81.91%.[34] Additionally, when the number of retrieved oocytes exceeds 20, there is a higher likelihood of excess oocytes, increased gonadotropin consumption, longer ovarian stimulation duration, and a significant portion of embryos remaining unused. To optimize IVF outcome, it is crucial to seek a balance between obtaining an optimal number of oocytes through an appropriate ovarian stimulation protocol and enhancing the quality of oocytes and embryos to achieve higher live birth rates. Although a decreased number of retrieved oocytes was noted in the present study, we observed an elevated fertilization rate and usable embryo rate. Importantly, there was no significant difference in the number of usable embryos and good-quality embryos between the groups, indicating a potential improvement in the quality of oocytes and embryos during letrozole co-treatment.

Pregnancy and Live Birth Rates

The available data for letrozole co-treatment in PCOS patients or high responders are limited.[31] A randomized parallel controlled study involving 130 individuals has revealed a negative impact on clinical pregnancy rate following the administration of letrozole in antagonist protocol for high responders, defined as those with an AFC of at least 15.[35] It may be attributed to the premature rise in progesterone levels during the late follicular phase. Another retrospective study with 125 non-obese PCOS participants has demonstrated a slightly favorable effect of letrozole on cumulative clinical pregnancy rate, but the evidence is not fulfilled the criteria as a high-quality study.[36] A recent retrospective study has shown that letrozole co-treatment with the progestin-primed ovarian stimulation protocol in PCOS patients resulted in a higher implantation rate, including all FET cycles associated with the IVF cycle.[37] According to previous studies, the

fully developed preovulatory-stage follicles promoted by letrozole are more likely to yield high-quality oocytes and embryos and lead to improved implantation potential.[38] It is important to note that prior studies have been limited by factors such as differences in patient populations, small sample sizes, variations in treatment protocols, and the use of different outcome measures not focused on live birth rates. In contrast, our study specifically examined the effects of letrozole co-treatment in women with PCOS undergoing IVF antagonist protocols, showing no significant differences between the letrozole and control groups in live birth outcomes after fresh and frozen-thawed ET cycles for women with PCOS. Nevertheless, future randomized controlled trials are needed to validate these findings.

OHSS Risks

PCOS patients are at a higher risk of developing OHSS during IVF. Lowering E2 levels with letrozole may have a potential clinical benefit in reducing the risk of OHSS.[11,39] A prior investigation has explored the dose-dependent reduction of serum E2 levels through the administration of letrozole, commencing on the day of oocyte retrieval in patients with high-risk OHSS.[40] It is proposed that continuing letrozole treatment after triggering, to stabilize E2 levels, may be a strategy to prevent or reduce the risk of OHSS.[40,41] Our study found a significantly lower level of E2 and a decreased number of follicles during letrozole cotreatment, as well as a higher rate of fresh ET, highlighting its potential application in lowering the risk of OHSS in women with PCOS. However, no conclusive evidence has been provided in such an effect, as most studies had limitations in sample size and power to assess OHSS as an outcome. In a previous meta-analysis of three studies, including 303 women (all normal responders), a non-significant tendency was observed towards a reduction of OHSS risk favoring letrozole.[31]

Obstetrical and Neonatal Complications

Letrozole co-treatment did not appear to be associated with any significant obstetrical or neonatal complications. Previous studies have also suggested that letrozole during IVF does not pose an elevated risk of major congenital anomalies or compromise neonatal outcomes compared to natural cycles, supporting the safety profile of letrozole.[42,43] This is reassuring and suggests that letrozole co-treatment may be a safe option for women with PCOS undergoing IVF/ICSI. However, further studies with larger sample sizes are needed to verify this notion.

Strengths and Limitations

This study was subject to several limitations that could potentially affect the generalizability and validity of its findings. The retrospective study design, for instance, might introduce selection bias due to its reliance on existing data rather than random assignment to treatment groups. Secondly, participants retrospective self-reporting of certain variables or outcomes might be subject to recall bias, potentially compromising the data accuracy. Despite statistical adjustments, residual confounding factors that were not accounted for in the analysis could still influence the results. Additionally, the study's constraints, such as a limited sample size for live births, lack of strict control over gonadotropin dosages, and failure to assess androgen levels, luteal phase hormone profiles, and endometrial receptivity, should be considered when interpreting the study findings.

Despite these limitations, the study made efforts to enhance its methodological robustness by utilizing high-quality data gathered from a single IVF center to ensure accurate outcome assessments and comprehensive collection of confounding variables, as well as employing a PSM model to reduce bias from confounding variables by mimicking randomization. Our study contributed to the literature by evaluating the endocrinological characteristics and clinical outcomes of letrozole co-administration in an IVF antagonist protocol for women with PCOS, filling a gap in the current research landscape.

Conclusion

Our study provides evidence that letrozole co-treatment in PCOS patients undergoing IVF/ICSI treatment is associated with a reduction in the number of retrieved oocytes but does not impact the number of embryos. Letrozole co-treatment results in a higher rate of fresh ET and does not have any significant adverse effects on pregnancy outcomes. The findings highlight the complex hormonal changes induced by letrozole and their impact on follicular development and oocyte retrieval. This information may guide clinicians in making informed decisions for patients with PCOS to reduce the risks of OHSS and potentially enhance the likelihood of a successful fresh embryo transfer. Further research is warranted to

fully elucidate the mechanisms underlying these effects and to optimize the use of letrozole co-treatment in IVF protocols for women with PCOS.

Abbreviations

AFC, antral follicle count; BMI, body mass index; E2, oestradiol; ET, embryo transfer; FET, frozen embryo transfer; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; hCG, human chorionic gonadotropin; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; LH, luteinizing hormone; OHSS, ovarian hyperstimulation syndrome; P, progesterone; PCOS, polycystic ovary syndrome; PSM, propensity score-based patient-matching.

Data Sharing Statement

All of the study's data are included in the publication and the Supplementary Information Files.

Ethical Study Approval

The Institutional Review Board of Shanghai Ninth People's Hospital of Shanghai Jiao Tong University School of Medicine (Shanghai, China) approved this retrospective cohort study (No. SH9H-2021-T467-2).

Informed Consent

Due to the deidentification of data and the retrospective nature of all analyses, the requirement for informed consent was waived by the Institutional Review Board of Shanghai Ninth People's Hospital.

Funding

This study was funded by the National Natural Science Foundation of China (Grant Nos. 82271693 and 82201912), and the Shanghai Sailing Program (21YF1423200).

Disclosure

The authors declare no conflicts of interest in this work.

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