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ORIGINAL RESEARCH

The Follicular Output Rate was Improved with 3-Day Letrozole Administration Compared with 5-Day Letrozole Administration Under Progestin-Primed Ovarian Stimulation

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Purpose: Progestin-primed ovarian stimulation (PPOS) has been widely employed in in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles. In recent years, letrozole (LE) combined with medroxyprogesterone acetate (MPA) has been used in this protocol to enhance ovarian response. This study compared the effects of a 5-day regimen with those of a 3-day regimen of letrozole within PPOS, focused on the follicular output rate (FORT) and blastocyst formation rates.

Patients and Methods: From January 2017 to January 2020, 1,754 infertility patients who received PPOS protocol were divided into two groups: 577 patients received 2.5 mg/day LE for 5 days (LE 5-day), and 1177 patients received the same dose of LE for 3 days (LE 3-day). Propensity score matching (1:1) balanced confounders, yielding 489 patients per group. The primary outcoms was the FORT. The rate of blastocyst formation was evaluated as the secondary outcome. A multivariable logistic regression analysis was performed to compare the disparity in the FORT between the two groups.

Results: After matching, the number of oocytes retrieved, number of mature oocytes, number of blastocysts, blastocyst formation rates, FORT, and clinical pregnancy rates were more favourable in the LE 3-day group than in the LE 5-day group ($P < 0.05$). In the multivariable linear regression model, after making adjustments for factors such as age, anti-Müllerian hormone (AMH), antral follicle count (AFC), body mass index (BMI), infertility type, and basal P, patients in the LE 3-day group exhibited an increase in the FOTR ($\beta = 0.08$, 95% confidence interval [CI] 0.02 to 0.14, $P = 0.0082$) and blastocyst formation rate ($\beta = 0.23$, 95% CI 0.17 to 0.29, $P < 0.0001$) compared to those in the LE 5-day group.

Conclusion: Compared with LE administration for 5 days, LE administration for 3 days may increase the FORT and the rate of blastocyst formation.

Keywords: in vitro fertilization, follicular output rate, letrozole, medroxyprogesterone acetate, ovarian stimulation

Introduction

In the field of assisted reproductive technology (ART), the process of controlled ovarian stimulation (COS) plays a vital role in facilitating the development of multiple follicles. Progestin-primed ovarian stimulation (PPOS) is a straightforward and efficient protocol for ovarian stimulation during controlled ovarian stimulation (COS) in the context of in vitro fertilization (IVF). This approach utilizes medroxyprogesterone acetate (MPA) as an oral alternative to

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prevent a premature luteinizing hormone (LH) surge.[1] Even though the PPOS protocol has had good results thus far, the feedback that comes from the progestogen can cause a persistent decline in LH levels, thus resulting in an increased dose of Gn and even follicular maldevelopment.

Letrozole (LE), a third-generation inhibitor of aromatase, effectively decreases the levels of oestrogen in both ovarian granulosa cells and the serum by hindering the conversion process from androgens to oestrogens.[2] LE is used during COS to reduce the total dose of exogenous FSH and the number of injections.[3] Research has been conducted on the utilization of letrozole within the PPOS protocol among individuals who have both polycystic ovary syndrome (PCOS) and obesity.[4] Our prior research investigated the simultaneous use of LE and MPA in ovarian stimulation for IVF. Our findings suggested that, compared with the PPOS protocol, the LE+PPOS protocol might be a viable option for women undergoing COS, potentially resulting in improved pregnancy results.[5] However, the duration of LE administration ranges from 3 to 5 days, and whether the different durations of LE in PPOS may affect the outcomes of COS has not been studied.

The FORT, which was first proposed in 2011[6], refers to the ratio of follicles that mature before ovulation, after follicle-stimulating hormone (FSH) stimulation, to the number of FSH-sensitive follicle reserves. The FORT can be used as a quantitative indicator of follicle development potential, independent of the original antral follicle count (AFC); it is related to the overall health of the follicles and is an objective indicator of ovarian responsiveness.[6] Studies have shown that the FORT is significantly positively correlated with clinical pregnancy outcomes and that patients with a high FORT have more mature oocytes, more blastocysts, and higher pregnancy rates, which indicates that the FORT can be used as an indicator to evaluate FSH responsiveness and the reproductive ability and predict IVF outcomes.[7,8]

After the incorporation of letrozole into the PPOS protocol, attempts have been made to explore both letrozole 5-day[5] (LE 5-day) and letrozole 3-day[9] (LE 3-day) regimens, both of which have favourable effects on ovulation induction. In this research, we present findings from a retrospective cohort study utilizing propensity score matching (PSM) to compare the FORTs between LE 3-day and LE 5-day administration in PPOS. Additionally, we offer recommendations for clinical practitioners on the basis of our results.

Materials and Methods

Study Design and Patients

This retrospective cohort study took place at the Renmin Hospital's Reproductive Medicine Center affiliated with Hubei University of Medicine and included females who underwent IVF/ICSI procedures from January 2017 to January 2020. The Ethics Committee of the Renmin Hospital, Hubei University of Medicine, approved the study (Approval No. SYSRMYY006).

The inclusion criteria were women aged between 20 and 50 years, experiencing infertility for a minimum of one year, having menstrual cycles that fall within the range of 25 to 35 days, and who have undergone IVF or ICSI.

The exclusion criteria were a history of any cancer, metabolic disorders such as thyroid disease, pelvic tuberculosis, congenital uterine malformations, chromosomal abnormalities, single-gene disorders, and immunological diseases.

The patients were categorized into two groups based on the duration of letrozole administration as follows: the LE 5-day group was administered a dosage of letrozole of 2.5 mg/day for 5 consecutive days during PPOS, and the LE 3-day group received letrozole at the same dosage but for only 3 straight days during PPOS.

Controlled Ovarian Stimulation

The process of ovarian stimulation was initiated on the 3rd day of menstruation in all patients. The LE 5-day group underwent ovarian stimulation with gonadotropin (Gn), and letrozole was administered from the first to the fifth day. The LE 3-day group, on the other hand, received Gn and letrozole for ovarian stimulation from the initial day until the third day. MPA was added from Day 4 of ovarian stimulation until the trigger day in both groups (Figure 1).

Follicle monitoring commenced on the third day of menstruation and was conducted every 2–4 days via transvaginal ultrasound assessment to document the quantity of developing follicles. Blood tests were performed concurrently with ultrasound examinations to measure the serum levels of FSH, LH, oestradiol (E2), and progesterone (P). Once the

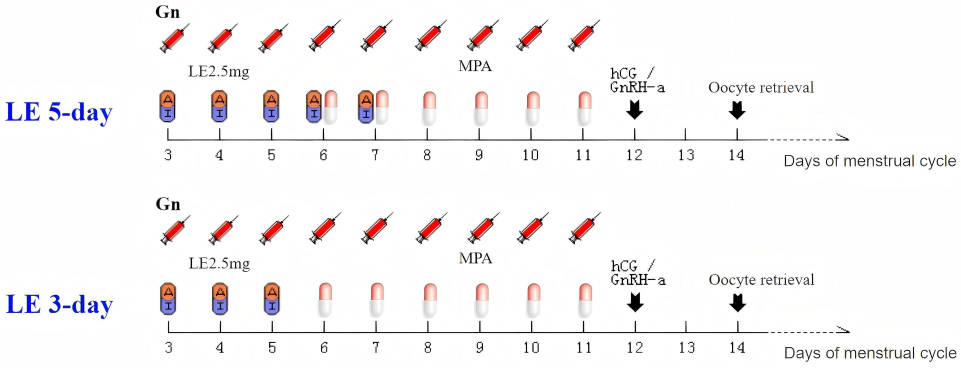

Figure 1 Treatment strategies in the two groups.
Abbreviations: LE, letrozole; MPA, medroxyprogesterone acetate; Gn, gonadotropin; hCG, human chorionic gonadotropin; GnRHa, gonadotropin releasing hormone analog.

follicles reached a diameter of 18 mm, triggering agents, including triptorelin (0.1 mg; Decapeptyl, Ferring Pharmaceuticals) and human chorionic gonadotropin (hCG; 2000 U, Lizhu Pharmaceutical Trading Co)., were administered to initiate the final stage of oocyte maturation.

The retrieval of oocytes was performed under the guidance of transvaginal ultrasound 34–36 hours after the trigger administration. All follicles with a diameter exceeding 10 mm were collected. IVF or ICSI techniques were employed on the basis of semen parameters for the fertilization of the retrieved oocytes. The evaluation of embryo quality was conducted on the third day postretrieval, and factors such as the blastomere quantity, distribution, and degree of fragmentation were considered. Embryo grading followed Cummin's[10] criteria, with high-quality embryos (grade-1 and grade-2 6-cell embryos and above) being cryopreserved via vitrification methods. Embryos that reached or exceeded three in number were further cultured until they developed into blastocysts by either Day 5 or Day 6 before undergoing freezing procedures.

Endometrial Preparation and FET

In this study, embryo and endometrium synchronization with frozen-thawed embryo transfer (FET) was performed via the same method in the two groups. The preparation method for FET included gonadotropin-releasing hormone analogue (GnRHa) downregulation with hormone replacement for all patients. For all patients, hormone treatment was recommended for endometrial preparation with oestradiol valerate (2 mg bid, Bayer Medical and Health Co). from the 3rd day of menstruation onwards. Sixteen days after the endometrial lining of each patient was confirmed to be thicker than 7 mm, dydrogesterone tablets (Abbott Biologicals B.V). were administered orally (2 tablets once a day), and progesterone injections (60 mg/day; Zhejiang Xianju Pharmaceutical Co). were also administered. The embryo transfer (ET) on Day 3 was scheduled to take place after three days. The blastocysts were transferred on the fifth day. Upon successful pregnancy, the administration of exogenous oestrogen and P supplements continued until the completion of 10 weeks of gestation.

Outcomes and Data Collection

In this study, the characteristics of ovarian stimulation, oocytes and embryos and pregnancy outcomes of frozen-thawed embryos from patients, including the sex hormone (FSH, LH, E_2, and P) levels on the starting day, the total gonadotropin dose, the duration of stimulation, the total dose of MPA, the sex hormone (FSH, LH, E_2, and P) levels on the hCG day, the number of oocytes retrieved, the number of mature oocytes, the number of fertilizations, the number of good-quality embryos, the number of transferred embryos, the type of the embryo transferred, the clinical pregnancy rates, and the live birth rates, were evaluated. The FORT was calculated as the ratio of the preovulatory follicle (16–22 mm in diameter)

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count to the dhCG × 100/small antral follicle (3–8 mm in diameter) count at baseline. The blastocyst formation rate was calculated as the ratio of the number of available blastocysts to the number of blastocysts cultured.

The definition of clinical pregnancy involved the identification of an intrauterine gestational sac through ultrasound examination 30 days after embryo transfer. Live birth was determined by the successful delivery of a living foetus after 28 weeks of gestation. The primary focus was on the rate of the first observed foetal heartbeats, whereas the secondary measure considered the percentage of blastocysts formed.

Statistical Analysis

Statistical analyses were conducted via SPSS version 22.0 and EmpowerStats (http://www.empowerstats.com; X&Y Solutions, Inc., Boston, Massachusetts, USA). The distribution of continuous variables was assessed via the Kolmogorov–Smirnov test. Variables that followed a normal distribution are reported as the means ± standard deviations and were assessed via the independent-sample *t* test. Variables that did not conform to a normal distribution are presented as medians (interquartile ranges (IQRs)) and were analysed via the Mann–Whitney *U*-test. Categorical variables are expressed as n (%) and were evaluated via either the chi-square test or Fisher's exact test.

To identify a group of patients with similar baseline characteristics, propensity score matching (PSM) was employed. The propensity score for utilizing different protocols was established on the basis of age, body mass index (BMI), anti-Müllerian hormone (AMH) level, and antral follicular count (AFC). PSM was conducted via a 1:1 matching protocol without replacement (a greedy matching algorithm) and a calliper width that was equivalent to 0.01 standard deviations of the logit of the propensity score. Conditional logistic regression analysis was subsequently used to assess the associations between the two groups and the FORT in a post-PSM population. A P level below 0.05 denoted statistical significance.

Results

Patient Characteristics

A total of 1754 cycles were included in the analysis. The process of data selection is depicted in Figure 2. After excluding certain cases, the eligible cohort comprised 577 women in the LE 5-day group and 1177 women in the LE 3-day group. Additionally, equal numbers of patients (489) were matched via PSM for each group (Table 1). Following PSM, no further changes were made.

Ovarian Stimulation Characteristics

After PSM, there were obvious differences between the groups in the total doses of gonadotropin, durations of stimulation, total doses of MPA, E_2 and P levels on the hCG day, numbers of oocytes retrieved, FORTs, numbers of mature oocytes, numbers of blastocysts, and blastocyst formation rates ($P<0.05$). However, there were no statistically significant differences between the two groups in terms of the basal hormone (FSH, LH, E_2, and P), FSH, and LH levels on the hCG day; the numbers of fertilizations; and the numbers of viable embryos obtained ($P > 0.05$). No moderate/severe OHSS occurred in either group (Table 2).

Figure 3 illustrates the hormonal changes in response to ovarian stimulation, specifically those in the FSH, LH, E_2, and P levels. In the LE 5-day group, there were significant increases in the FSH levels three days later; however, the levels then remained stable until the trigger day. However, in the LE 3-day group, the FSH levels stabilized after five days. There were no notable differences in the FSH levels between the two groups at any given time (Figure 3A). During COS, LH levels on Days 4 and 6 were considerably lower in the LE 3-day group than in the LE 5-day group (P <0.01). Neither group experienced a premature LH surge (Figure 3B). As the follicles matured, the serum E_2 level steadily increased. From Day 6 to the trigger day, the oestrogen values were significantly greater in the LE 3-day group than in the LE 5-day group (P <0.01) (Figure 3C). On the trigger day, P levels were substantially lower in the LE 3-day group than in the LE 5-day group (P<0.01) (Figure 3D).

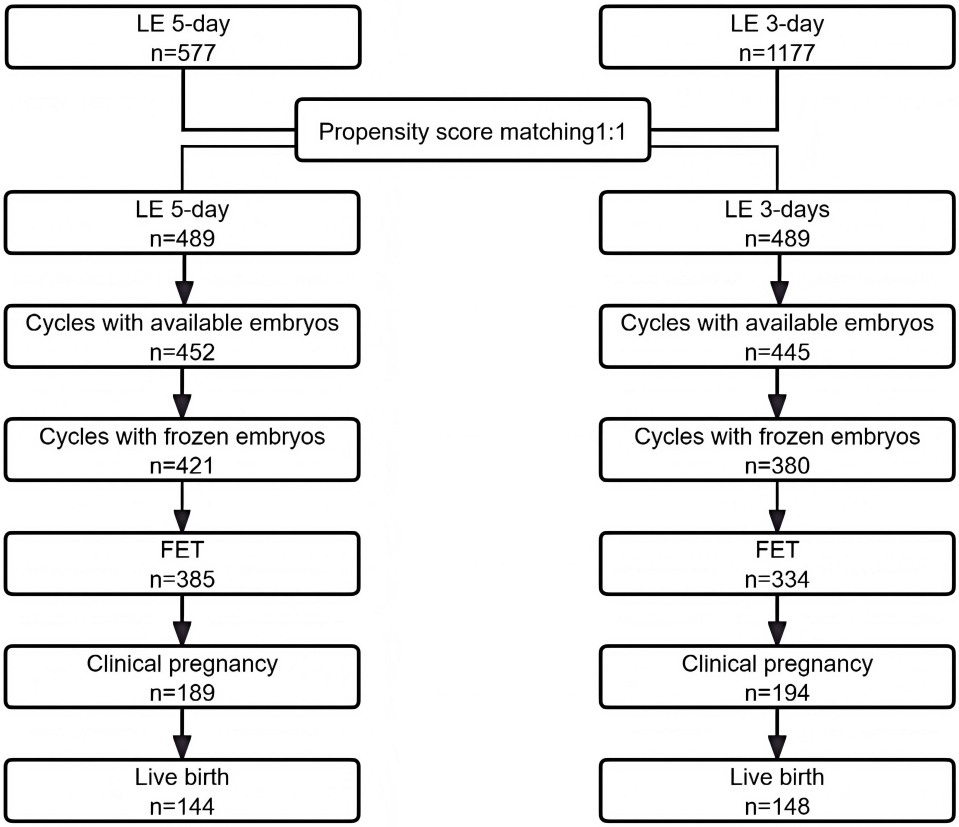

Figure 2 Flow chart of the study.

Pregnancy Outcomes of Frozen-Thawed Embryos

The outcomes are presented in Table 3. Before and after PSM, the clinical pregnancy rates and blastocyst transfer rates in the LE 3-day group were significantly higher than those in the LE 5-day group (P<0.05). Moreover, the number of

Table 1 Basic Characteristics of the Patients in the Two Groups Before and After Propensity Score Matching

	Before Propensity Matching		*P*	After Propensity Matching		*P*
	LE 5-Day	LE 3-Day		LE 5-Day	LE 3-Day	
No. of cycles	577	1177		489	489	
Age (years)	35.32 (5.82)	33.74 (5.28)	<0.001	34.98 (5.65)	34.56 (5.46)	0.2360
Infertility Duration (years)	4.70 (4.06)	4.19 (3.50)	0.007	4.65 (4.02)	4.27 (3.70)	0.1162
Antral follicular count	6.11 (3.21)	7.12 (3.27)	<0.001	6.33 (3.18)	6.56 (3.04)	0.2457
BMI (kg/m2)	22.93 (3.37)	23.46 (3.52)	0.003	22.99 (3.44)	22.96 (3.24)	0.9234
AMH (ng/mL)	2.14 (1.91)	2.37 (1.90)	0.025	2.11 (1.81)	2.16 (1.61)	0.6326
Infertility type, n (%)			<0.001			0.0500
Primary	193 /577 (33.80)	506 /1177 (42.99)		168 (34.4)	196 (40.1)	
Secondary	378 /577 (66.20)	671 /1177 (57.01)		321 (65.6)	293 (59.9)	
Infertility factors, n (%)			<0.001			0.1288
Pelvic and tubal factors	400 /577 (69.32)	648 /1177 (55.06)		335 (68.5)	319 (65.2)	
Ovulation disorder	87 /577 (15.08)	263 /1177 (22.34)		76 (15.5)	102 (20.9)	
Endometriosis	39 /577 (6.76)	82 /1177 (6.97)		34 (7)	31 (6.3)	
Male factor	44 /577 (7.63)	160 /1177 (13.59)		37 (7.6)	26 (5.3)	
Unexplained	7 /577 (1.21)	24 /1177 (2.04)		7 (1.4)	11 (2.3)	

Notes: Date: mean (SD) or (%) (no./total no).
Abbreviations: BMI, body mass index; AMH, anti-Müllerian hormone.

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Table 2 Ovarian Stimulation Characteristics in the Two Groups Before and After PSM

	Before Propensity Matching			After Propensity Matching		
	LE 5-Day	**LE 3-Day**	***p***	**LE 5-Day**	**LE 3-Day**	***p***
Basal FSH (IU/L)	8.06 (3.98)	7.56 (2.95)	0.526	7.90 (3.68)	7.75 (3.12)	0.4981
Basal LH (IU/L)	5.09 (3.14)	4.94 (3.25)	0.371	5.05 (3.07)	4.89 (3.11)	0.4208
Basal E2 (pg/mL)	44.37 (21.97)	45.30 (21.38)	0.444	44.74 (21.15)	46.17 (22.37)	0.3565
Basal P(pg/mL)	0.67 (0.45)	0.62 (0.43)	0.015	0.68 (0.45)	0.62 (0.43)	0.0531
Total dose of gonadotropin (IU)	1708.97 (565.28)	2061.05 (568.38)	<0.001	1729.79 (556.68)	2056.83 (561.64)	<0.0001
Duration of stimulation (days)	9.31 (1.81)	10.10 (1.55)	<0.001	9.42 (1.71)	10.13 (1.55)	<0.0001
Total dose of MPA (mg)	62.89 (16.61)	53.05 (11.94)	<0.001	63.75 (16.28)	53.58 (11.95)	<0.0001
FSH on hCG day (IU/L)	18.27 (5.18)	18.73 (6.93)	0.863	18.41 (5.27)	19.49 (0.65)	0.773
LH on hCG day (IU/L)	3.31 (1.96)	3.01 (1.77)	0.002	3.31 (1.91)	3.19 (1.85)	0.3192
E_2 on hCG day (pg/mL)	1015.89 (788.76)	1848.86 (1239.79)	<0.001	1006.56 (712.77)	1738.36 (1056.21)	<0.0001
P on hCG day (ng/mL)	0.87 (0.42)	0.78 (0.39)	<0.001	0.87 (0.42)	0.79 (0.39)	0.002
Oocyte retrieved	5.03 (3.06)	6.07 (2.89)	<0.001	5.11 (2.96)	5.64 (2.63)	0.0033
FORT	0.73 (0.48)	0.76 (0.49)	0.009	0.73 (0.48)	0.81 (0.54)	0.0187
No. of mature oocytes	4.31 (2.82)	5.17 (2.76)	<0.001	4.46 (2.76)	4.83 (2.50)	0.0299
No. of fertilization	4.01 (2.64)	6.64 (2.17)	<0.001	4.17 (2.60)	6.50 (2.12)	0.2048
No. of useful quality embryos	2.29 (1.45)	2.40 (1.42)	0.144	2.38 (1.41)	2.27 (1.38)	0.232
No. of blastocysts	0.49 (1.17)	2.34 (1.29)	<0.001	0.49 (1.17)	2.34 (1.29)	<0.001
Blastocyst formation rate (%)	285/447(63.76)	1946/2587(75.22)	<0.001	264/404(65.35)	753/1029(73.18)	<0.001
Moderate/severe OHSS, n (%)	0	0	/	0	0	/

Notes: Date: mean (SD) or (%) (no./total no).

Abbreviations: FSH,follicular-stimulating hormone; LH, luteinizing hormone; E_2, estradiol; P, progesterone; MPA, medroxyprogesterone acetate; hCG, human chorionic gonadotrophin; FOTR, follicular output rate; OHSS, ovarian hyper-stimulation syndrome.

transferred embryos was lower in the LE 3-day group than in the LE 5-day group. The endometrial thickness on the day of ET and the live birth rates were comparable between the two groups (P>0.05) (Table 3).

Multivariable Analysis of Outcomes Before and After PSM

The results of the multivariable analysis indicated that LE 3-day administration resulted in significantly greater FORT (OR=0.08, 95% CI: 0.02–0.14, P=0.0082) and blastocyst formation rate (OR=0.23, 95% CI: 0.17–0.29, P<0.0001) than LE 5-day administration did after accounting for various factors, such as age, AMH level, AFC count, BMI, infertility type, and basal P level. After adjusting for age, AMH, AFC, BMI, infertility type, basal P level, type of transferred embryo, and number of transferred embryos, the clinical pregnancy rates were found to be comparable between the two groups after PSM. (Table 4).

Discussion

In this retrospective cohort study utilizing propensity score matching among 1754 women who underwent IVF/ICSI, the FORT was significantly greater in the LE 3-day group than in the LE 5-day group.

As early as 2006, incorporating LE into gonadotropin (Gn) administration was proposed to lead to an increase in the number of preovulatory follicles.[11] The FORT, which can be utilized to assess the ovarian response to exogenous follicle-stimulating hormone (FSH) in infertile patients, is calculated as the ratio of the number of preovulatory follicles that are generated in response to the administration of FSH to the preexisting pool of small antral follicles.[12] LE administration in the PPOS protocol was shown to effectively improve the FORT among women diagnosed with PCOS and having a high BMI.[4] These findings collectively suggest that LE positively impacts ovarian responsiveness. Since its introduction along with PPOS at our centre in 2017, multiple studies have consistently affirmed LE benefits in terms of oocyte retrieval numbers[9] and even clinical pregnancy rates.[5]

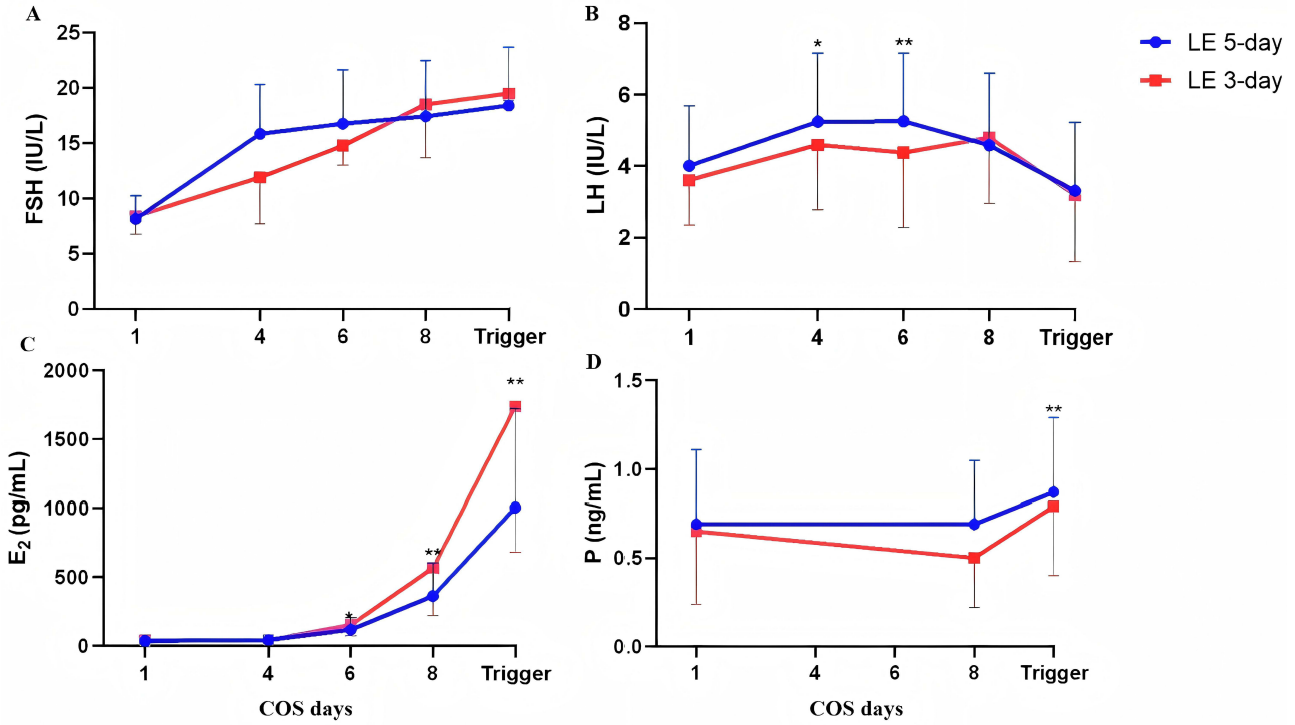

Figure 3 The dynamic changes in hormones during ovarian stimulation in the two groups. **(A)** Serum FSH levels, **(B)** LH concentration, **(C)** E_2 levels, and **(D)** P levels in the two groups during COS. The asterisks denote significant changes in hormone levels (*P<0.05, **P<0.01).
Abbreviations: COS, controlled ovarian stimulation; FSH, follicle stimulating hormone; LH, luteinizing hormone; E2, estrogen; P, progesterone.

Although the effect of LE (2.5 mg/day in most centres) in combination with PPOS has been recognized by many researchers, no consensus has been reached on the number of days to use LE, and whether differences in the duration of LE administration affect ovulation induction is unknown. To maximize the benefits of ovarian stimulation, we used LE for 5 and 3 days and were surprised to find that LE administered for 3 days resulted in higher FORT and blastocyst formation rate. We hypothesized that the following three reasons might explain these results:

First, the decrease in the LE duration by 2 days resulted in an improvement in follicular synchrony. Letrozole, a third-generation aromatase inhibitor (AI), preserves the integrity of central feedback mechanisms by not antagonizing oestrogen receptors in the brain. The commencement of follicular development, accompanied by increasing levels of oestrogen hormones, establishes a typical feedback loop that limits the FSH response and inhibits the degeneration of

Table 3 Pregnancy Outcomes of Frozen-Thawed Embryos in the Two Groups Before and After PSM

	Before Propensity Matching			After Propensity Matching		
	LE 5-Day	LE 3-Day	*p*	LE 5-Day	LE 3-Day	*p*
No. of cycles	435	845		385	334	
No. of transferred embryos	2.02 (0.49)	1.58 (0.51)	<0.001	2.02 (0.50)	1.57 (0.51)	<0.001
Endometrial thickness on ET day(mm)	10.34 (2.75)	10.30 (2.37)	0.800	10.38 (2.73)	10.23 (2.33)	0.4664
The type of embryo transferred						<0.0001
Cleavage-stage embryo (%)	299 (68.89)	91 (10.78)	<0.001	258 (67.4)	32 (9.6)	
Blastocyst (%)	135 (31.11)	753 (89.22)		125 (32.6)	301 (90.4)	
Clinical pregnancy rates (%)	209/435 (48.05)	503/845 (59.53)	<0.001	189 /385 (49.1)	194 /334 (58.1)	0.0195
Live birth rates (%)	161 (77.03)	396 (78.73)	0.231	144 (76.2)	148 (76.3)	0.8488

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Table 4 Multivariable Analysis of Outcomes Before and After PSM

Exposure	Before Propensity Matching		After Propensity Matching	
	Non-Adjusted	Adjust I	Non-Adjusted	Adjust II
FOTR				
LE 5-day	0	0	0	0
LE 3-day	0.03 (−0.01, 0.08) 0.1651	0.08 (0.03, 0.13) 0.0011	0.08 (0.01, 0.14) 0.0187	0.08 (0.02, 0.14) 0.0082
Blastocyst formation rate				
LE 5-day	0	0	0	0
LE 3-day	0.22 (0.17, 0.27) <0.0001	0.24 (0.19, 0.29) <0.0001	0.19 (0.14, 0.25) <0.0001	0.23 (0.17, 0.29) <0.0001
Clinical pregnancy rates				
LE 5-day	1	1	1	1
LE 3-day	1.59 (1.26, 2.01) <0.0001	0.76 (0.51, 1.15) 0.1927*	1.44 (1.07, 1.93) 0.0161	0.76 (0.47, 1.24) 0.2738*

Notes: Data was shown as b (95% CI) P value /OR (95% CI) P value. Non-adjusted model adjusts for: None. * Adjust I model and Adjust II model were adjusted for age, AMH, AFC, BMI, Infertility type, Basal P, type of embryo transferred, and number of transferred embryos.
Abbreviation: FOTR, follicular output rate.

small follicles, ultimately resulting in single-ovulation cycles.[13] Consequently, we postulated that an extended duration of LE administration would correlate with an increased probability of follicle optimization. LE significantly inhibits oestradiol after 2 to 3 days of administration, and its half-life is 45 hours.[14] When the duration of LE administration is reduced by 2 days, the concentration of the drug in the body decreases rapidly, thereby attenuating its adverse effects of limiting dominant follicle development. In monovulatory species, the follicles of each major follicular wave experience a synchronized period of growth that lasts for 2 or 3 days after the peak of the FSH surge is reached. Following this growth phase, diameter deviation is initiated, which serves as a morphological indication for follicle selection.[15] The administration of LE for a duration of 3 days coincided with the period of simultaneous growth of follicles, thereby augmenting the effect of follicular cogrowth and avoiding, upon timely discontinuation, the differentiation of follicles.

Second, LH plays a crucial role in follicle dominance selection.[16] Follicle diameter deviation occurs close to the nadir during the wave-induced surge of follicle-stimulating hormone (FSH),[17] and the occurrence of this event aligns with various modifications in gene expression within granulosa cells of the future dominant follicle. These alterations include an increase in the mRNA level of CYP19A, resulting in increased production of follicular insulin-like growth factor 1 (IGF-1). Additionally, there is an increase in the level of the mRNA encoding PAPPA, a protease responsible for the degradation of IGF-binding proteins, which leads to elevated levels of free IGF-1 in the follicular fluid. Furthermore, luteinizing hormone receptor (LHr) mRNA is augmented. Basic experiments revealed the suppression of follicle diameter deviation following LH inhibition.[16] Considering the fluctuations in hormone levels during ovarian stimulation observed in both groups, the LH levels were significantly lower in the LE 3-day group than in the LE 5-day group on Days 4 and 6 of ovarian stimulation, which further contributed to the proliferation of additional follicles, consequently leading to the FORT increase.

Third, by reducing the duration of LE use, the duration of Gn use was extended when both endogenous and exogenous Gn doses were sufficient, providing an opportunity for more follicle growth, higher late follicular phase E_2 levels, and a greater number of oocytes retrieved in the LE 3-day group. The coadministration of LE led to a reduction in oestrogen levels and caused increases in the accumulation of P, 17α-progesterone, and testosterone.[18–20] These alterations in the endocrine microenvironment likely impact the process of meiotic maturation by impeding the development of the oocyte cytoplasm, thereby leading to a decrease in oocyte maturation. Therefore, we speculated that the reduction of LE use by 2 days in the LE 3-day group might be helpful for oocyte maturation. Once the number of mature oocytes increases, this leads to increased potential for blastocyst culture, elevated rates of blastocyst formation, and increased quantity of blastocysts. Consequently, more blastocysts are transferred during cycles, resulting in an increased clinical pregnancy rate.

This research stands out because of the utilization of PSM and the administration of LE for varying durations while maintaining a consistent dosage. This allowed us to thoroughly investigate the benefits and drawbacks associated with

administering LE over either 3 or 5 days. However, it is important to acknowledge that the retrospective design of our study could introduce limitations. Despite matching patients on the basis of propensity, there remained a possibility of individual variances that could impact our research findings. Another limitation was a partial lack of progesterone during COS; therefore, a comprehensive evaluation of the trends in serum progesterone levels resulting from the two regimens was not feasible. The inclusion of all patients in embryo transfer would be essential for obtaining accurate data, as there were individuals in each group who did not undergo embryo transfer. The current data may be prone to bias when evaluating pregnancy outcomes. Furthermore, further research on the molecular mechanism of follicular dominance in these two protocols is necessary. Additionally, no comparison was made regarding the economic and time implications associated with both approaches.

Conclusion

In summary, the findings of this study indicate that the administration of LE for 3 days may lead to a significantly greater FORT than the LE 5-day method during PPOS. Additionally, the blastocyst formation rate improved with the LE 3-day protocol. One possible explanation for these superior outcomes with LE administration for 3 days could be attributed to its positive impact on hormone levels and synchronization of follicular development. To comprehensively evaluate the advantages and disadvantages of both protocols, further well-designed randomized controlled trials are warranted.

Data Sharing Statement

All data presented in this study are available upon request and contact with the corresponding author.

Ethics Approval and Consent to Participate

This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Renmin Hospital, Hubei University of Medicine (Approval No. SYSRMYY006). The requirement for written informed consent was waived by the Ethics Committee due to the retrospective nature of the study and the use of anonymized clinical data. All patient information was strictly de-identified prior to analysis to ensure confidentiality, and data access was restricted to authorized researchers in compliance with institutional guidelines.

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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 competing interests.

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