Liang *et al.*
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Differential optimal follicle sizes for ovulatory dysfunction and unexplained infertility in LE-IUI cycles: a retrospective analysis

Yihua Liang[1,2], Haiyan Lin[1,2], Qi Qiu[1,2], Ping Pan[1,2], Xuedan Jiao[1,2], Yu Li[1,2*] and Qingxue Zhang[1,2*]

Abstract

Background This study aims to identify the optimal dominant follicle size on the trigger day in patients with ovulatory dysfunction and unexplained infertility undergoing intrauterine insemination with letrozole (LE-IUI) cycles.

Methods A retrospective analysis included 411 cycles of each group after 1:1 propensity score matching, comparing basic characteristics and outcomes based on dominant follicle size.

Results Higher rates of HCG positive, clinical pregnancy, and live birth were found in ovulatory dysfunction versus unexplained infertility (22.4% vs. 9.5%; 21.5% vs. 7.9%; 19% vs. 7.1%, $P < 0.001$). In ovulatory dysfunction, dominant follicles 17–18.9 mm had lower rates of HCG positive (7.6% vs. 21.5% vs. 26.2%, $P = 0.007$), clinical pregnancy (6.1% vs. 21.5% vs. 25.6%, $P = 0.004$), and live birth (4.5% vs. 19.2% vs. 23.2%, $P = 0.004$) compared to 19–21.0 mm and > 21.0 mm groups. Conversely, in unexplained infertility, follicles > 21.0 mm had lower HCG positive rate(13.3% vs. 11.8% vs. 3.4%, $P = 0.023$) compare to other two groups. Dominant follicle size on trigger day influenced HCG positive rate in LE-IUI cycles for both groups.

Conclusion Triggering at follicle size ≥ 19.0 mm may be optimal for ovulatory dysfunction, while a size ≤ 21 mm may improve HCG positive rates in unexplained infertility, underscoring the need to consider infertility factors in trigger decisions.

Trial registration: This study is registered with China Medical Research Online (Registration Number: MR-44-23-038090S, www.medicalresearch.org.cn).

Keywords Intrauterine insemination, Letrozole, Dominant follicle size, HCG positive rate

Background

Intrauterine insemination with ovulation induction (OI-IUI) represents a cost-effective protocol in infertility treatment, particularly for patients with ovulatory dysfunction and unexplained infertility. Letrozole (LE), a third-generation aromatase inhibitor, has become a significant treatment option since 2001 [1]. By inhibiting estrogen biosynthesis, LE disrupts estrogen feedback at the pituitary gland, thereby promoting serum FSH secretion and stimulating ovulation [2]. While the low

*Correspondence:
Yu Li
liyuliyu0922@163.com
Qingxue Zhang
zhqingx@mail.sysu.edu.cn
[1] Reproductive Medicine Centre, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou 510120, China
[2] Guangdong Provincial Clinical Research Center for Obstetrical and Gynecological Diseases, Guangzhou 510020, China

estrogen state induced by LE can lead to side effects such as hot flashes and nausea, these are relatively uncommon in reproductive field due to the short-term use and brief half-life of the drug [3]. The convenience of oral administration, effectiveness in ovulation induction, and minimal adverse effects on endometrial thickness have contributed to LE's widespread use as ovulation induction medicine.

To optimize the use of LE for ovulation induction, numerous clinical studies have been conducted. However, while the optimal dominant follicle size for triggering ovulation in LE-IUI cycles has garnered attention, research on this topic remains limited and inconclusive. For instance, Kuang et al. reported an optimal dominant follicle size between 16.1 and 18.0 mm in HCG-triggered LE-IUI cycles [4]. Conversely, another study observed peak pregnancy rates when triggering was initiated at a dominant follicle size of 19.1–21.0 mm [5]. Despite including a substantial number of cases, these studies lacked focus on specific infertility populations and varied in their letrozole protocols, leading to limitations in the applicability of their conclusions.

Ovulatory dysfunction accounts for approximately 25% of infertility diagnoses, while unexplained infertility affects about 15% of infertile couples [6]. At our medical center, these conditions even represent 50% of IUI patients, highlighting the need to improve IUI success rates in these populations. Our study seeks to identify optimal dominant follicle size on trigger day for LE-IUI in patients with ovulatory dysfunction and unexplained infertility by analyzing cycle outcomes through different follicle sizes.

Methods

Study design

This retrospective analysis was conducted at the Reproductive Medicine Centre of Sun Yat-sen Memorial Hospital from January 2016 to December 2022. We collected ultrasound and clinical data from couples with ovulatory dysfunction or unexplained infertility who underwent LE-IUI. The study was approved by the Ethics Committee of Sun Yat-sen Memorial Hospital (Approval Number: SYSKY-2023-1054-01) and is registered with China Medical Research Online (Registration Number: MR-44-23-038090S, www.medicalresearch.org.cn). As the study involved a retrospective analysis of anonymized patient data, explicit patient consent was not required.

Study population

The inclusion criteria for this study were: females under 40 years of age; confirmation of bilateral tubal patency via hysterosalpingography or laparoscopic surgery; normal semen analysis parameters in the male partner according to WHO guidelines; and a post-preparation total motile sperm count (TMSC) $\geq 10 \times 10^6$. Patients included in the study presented with either ovulatory dysfunction or unexplained infertility. Exclusion criteria encompassed basal FSH > 10 mIU/ml; previous ovarian or tubal surgeries; endometriosis or adenomyosis; history of intrauterine adhesions, uterine or cervical abnormalities; history of fetal anomalies or recurrent miscarriages, and previous malignancies.

Ovarian stimulation protocols

Letrozole (Zhejiang Haizheng Pharmaceutical, China) was administered orally at a dose of 2.5 or 5 mg daily on cycle days 3, 4, or 5 following a transvaginal ultrasound examination, and continued for 5 days. Subsequently, low doses of gonadotropins were added based on follicular development as needed. Transvaginal ultrasounds (TVS) were performed to monitor follicle growth after letrozole treatment and at intervals of 1–3 days. Trigger administration occurred when the dominant follicle's average diameter reached at least 18 mm or when the follicle reached 14 mm with elevated urinary LH levels. Cycles with dominant follicles smaller than 17 mm were excluded, as this was a rare occurrence. Ovulation was induced using human chorionic gonadotropin (HCG; Lizhu Pharmaceutical Trading Co., China), triptorelin (Ferring Pharmaceuticals, Switzerland; Changchun Jinsai Pharmaceuticals, China), or a combination of the two. IUI was performed within 24–36 h following trigger administration. In cases of an early LH surge with a dominant follicle diameter below 14 mm or the presence of more than three dominant follicles, the cycle was canceled.

Sperm preparation and insemination

On the day of IUI, the male partner provided a semen sample which underwent density gradient centrifugation according to our laboratory protocol. The density gradient centrifugation protocol was performed using a two-layer system (45%/90% SpermGrade). Liquefied semen was centrifuged through the gradients (500 g, 15 min), followed by washing (300 g, 5 min). The final sperm suspension (0.5 mL) was maintained at 35 °C, 6% CO_2 until use. All procedures were performed at room temperature under dual-operator verification. Approximately 0.5 ml of the sperm suspension was then introduced into the uterus using a soft catheter (Cook Group, USA; HUANHAO, Shenzhen, China). Following insemination, patients were instructed to remain in a supine position for at least 20 min. TVS was conducted 48 h post-IUI to confirm ovulation. Upon confirming ovulation, progesterone was administered for luteal support.

Main outcomes and statistical analysis

The primary outcome of this study was HCG positive rate. Patients underwent serum HCG testing 14–17 days after IUI, with a positive HCG result defined as a serum HCG concentration exceeding 25 mIU/ml. Clinical pregnancy was confirmed via TVS when at least one gestational sac was visible in the uterus three weeks post-positive HCG. Early miscarriage was characterized by either the absence of a detectable heartbeat in the gestational sac or a spontaneous miscarriage occurring within the first 12 weeks of gestation. A live birth was defined as the delivery of an infant after the 28th week of gestation, while multiple pregnancies were identified as those involving more than one fetus.

Statistical analysis was performed using the Statistical Package for the Social Sciences (version 25.0; SPSS Inc., Armonk, New York, USA). The normality of continuous variables was assessed using the Shapiro–Wilk test and histogram analysis. Continuous variables are presented as mean ± SD, while categorical variables are expressed as frequency and percentage. Group comparisons were conducted using either the Student's t-test or the chi-squared test as appropriate. Rates of HCG positive per IUI cycle were stratified by dominant follicle size in 1 mm intervals. Binary logistic regression analysis was employed to evaluate the effects of covariates on the HCG positive rate. A P-value of < 0.05 was considered statistically significant. Propensity score matching (PSM) was utilized to balance treatment groups based on confounding factors that may impact IUI outcomes. Potential confounding variables included female and male age, female BMI, duration of infertility, basal FSH level, and the number of follicles ≥ 14 mm and ≥ 16 mm. Subjects were matched 1:1 using nearest neighbor matching without replacement, in a random order, with a caliper value of 0.01. Prior to analysis, we conducted a thorough initial screening of the dataset to identify any missing values, and cycles with missing data were excluded from the final analysis.

Results

Comparative analysis of clinical outcomes in ovulatory dysfunction and unexplained infertility

A total of 693 cycles of ovulatory dysfunction and 580 cycles of unexplained infertility were initially screened for this study. Significant differences were observed in the age of males and females, female BMI, Basel FSH, Basel LH, duration of infertility between the two cohorts prior to matching ($P < 0.05$, Table 1).Patients with ovulatory dysfunction demonstrated markedly higher rates of HCG positive, clinical pregnancy, and live birth compared to those with unexplained infertility (22.4% vs. 9.5%; 21.5% vs. 7.9%; 19% vs. 7.1%, respectively, $P < 0.001$).Propensity score matching (1:1) was utilized to eliminate baseline characteristic disparities, resulting in 411 cycles in each group after matching. Post-matching, patients with ovulatory dysfunction continued to exhibit significantly higher basal LH levels, larger dominant follicle sizes, and a greater number of follicles measuring ≥ 18 mm ($P < 0.05$, Table 1). Moreover, the rates of HCG positive, clinical pregnancy, and live birth remained higher in the ovulatory dysfunction group (21.2% vs. 9.7%; 20.7% vs. 8.5%; 18.5% vs. 8.3%, respectively, $P < 0.001$). In terms of multiple pregnancies, the rate in the ovulatory dysfunction group was 5.9%, while it was 2.9% in the unexplained infertility group. The two rates are similar and there is no statistical significance in the comparison.

Clinical outcomes by dominant follicle size in different patient groups

We further categorized cycles of both patient types into three subgroups based on the size of the dominant follicle: 17–18.9 mm (Group A), 19–21.0 mm (Group B), and > 21.0 mm (Group C). The baseline characteristics and clinical outcomes for different dominant follicle sizes are presented in Tables 2 and 3.

Among patients with ovulatory dysfunction, no significant differences were observed across the three subgroups in terms of female and male age, female BMI, basal hormone levels, infertility type, duration of infertility, endometrial thickness at triggering, and the number of follicles ≥ 14.0 mm and ≥ 16.0 mm. However, Group A (17–18.9 mm) exhibited a lower number of follicles ≥ 18 mm and significantly lower rates of HCG positive (7.6% vs. 21.5% vs. 26.2%, $P = 0.007$), clinical pregnancy (6.1% vs. 21.5% vs. 25.6%, $P = 0.004$), and live birth (4.5% vs. 19.2% vs. 23.2%, $P = 0.004$) compared to the other two groups. Although Group A demonstrated a higher early miscarriage rate (25% vs. 8.1% vs. 9.3%, $P = 0.549$), this difference was not statistically significant.

For patients with unexplained infertility, no significant differences in baseline characteristics were observed among the three dominant follicle size groups. Notably, when the follicle size exceeded 21 mm (Group C), patients exhibited a significantly lower rate of HCG positive (13.3% vs. 11.8% vs. 3.4%, $P = 0.023$), contrasting with the trend observed in ovulatory dysfunction patients. Group C also demonstrated lower rates of clinical pregnancy (10.8% vs. 10.4% vs. 3.4%, $P = 0.065$) and live birth (9.6% vs. 10.4% vs. 3.4%, $P = 0.077$), although these differences did not reach statistical significance.

Figure 1 shows the HCG positive rates for patients with ovulatory dysfunction and unexplained infertility, categorized by 1-mm increments in dominant follicle size. Most patients were triggered with follicles sized between

Table 1 Basic characteristic and clinical outcomes in LE-IUI cycles for ovulatory dysfunction and unexplained infertility before and after propensity score matching ($\bar{x} \pm s$)

	Before matching			After matching		
	OD (n = 693)	UI (n = 580)	*P* value	OD (n = 411)	UI (n = 411)	*P* value
Female age (year)	30.35 ± 3.56	31.90 ± 3.43	< 0.001	31.02 ± 3.44	31.20 ± 3.43	0.452
Male age (year)	32.86 ± 3.99	33.82 ± 4.29	< 0.001	33.21 ± 3.90	33.37 ± 4.32	0.576
Basel FSH (mIU/ml)	6.96 ± 2.06	7.59 ± 2.00	< 0.001	7.21 ± 2.26	7.36 ± 1.89	0.29
Basel LH (mIU/ml)	8.40 ± 5.06	5.10 ± 2.65	< 0.001	8.61 ± 5.16	4.86 ± 2.22	< 0.001
Female BMI (kg/m^2)	22.36 ± 3.36	20.76 ± 2.47	< 0.001	20.85 ± 2.57	21.20 ± 2.57	0.051
Duration of infertility (years)	3.88 ± 2.12	3.22 ± 1.77	< 0.001	3.50 ± 1.96	3.43 ± 1.82	0.591
Infertility type, n (%)			0.025			0.318
Primary	515 (74.3)	398 (68.6)		298 (72.5)	285 (69.3)	
Secondary	178 (25.7)	182 (31.4)		113 (27.4)	126 (30.7)	
Dominant follicle size (mm)	20.68 ± 2.17	20.36 ± 1.84	< 0.001	20.97 ± 2.17	20.30 ± 1.79	< 0.001
Endometrial thickness on trigger day (mm)	9.65 ± 2.12	9.60 ± 2.06	0.629	9.65 ± 2.16	9.53 ± 1.99	0.423
Number of follicles ≥ 14 mm	1.48 ± 0.789	1.4 ± 0.659	0.062	1.47 ± 0.80	1.41 ± 0.67	0.237
Number of follicles ≥ 16 mm	1.32 ± 0.56	1.28 ± 0.54	0.309	1.36 ± 0.61	1.28 ± 0.55	0.082
Number of follicles ≥ 18 mm	1.09 ± 0.50	1.10 ± 0.45	0.751	1.18 ± 0.54	1.10 ± 0.47	0.023
TMCS (*10^6)	120.66 ± 93.28	114.44 ± 88.42	0.227	116.49 ± 90.28	114.44 ± 88.66	0.744
HCG positive rate, n (%)	155 (22.4)	55 (9.5)	< 0.001	87 (21.2)	40 (9.7)	< 0.001
Clinical pregnancy rate, n (%)	149 (21.5)	46 (7.9)	< 0.001	85 (20.7)	35 (8.5)	< 0.001
Live birth rate, n (%)	132 (19.0)	41 (7.1)	< 0.001	76 (18.5)	34 (8.3)	< 0.001
Multiple pregnancy rate, n (%)	10 (6.7)	1 (2.2)	0.344	5 (5.9)	1 (2.9)	0.591
Early miscarriage rate, n (%)	16 (10.7)	5 (10.9)	1.00	8 (9.4)	1 (2.9)	0.215

OD: ouvlatory dysfunction; UI: unexplained infertility; TMCS: the post-preparation total motile count of sperm

P < 0.05 was considered statistically significant

18 and 22.9 mm. In patients with unexplained infertility, the HCG positive rate declined as dominant follicle size increased, while no such trend was observed in those with ovulatory dysfunction. Notably, no pregnancies occurred in the unexplained infertility group when follicle size exceeded 23 mm. HCG positive rates for each dominant follicle size are detailed in Supplemental Tables 1 and 2, highlighting the differing impacts of follicle size on treatment outcomes between the two groups.

Factors influencing HCG positive rate: binary logistic regression analysis

Binary logistic analysis was performed to evaluate factors influencing the HCG positive rate. Tables 4 and 5 present the univariable and multivariable results. For ovulatory dysfunction (Table 4), female age and dominant follicle size group were significantly correlated with HCG positive rate in the adjusted model. Female age showed a negative correlation (adjusted OR 0.892, 95% CI 0.828–0.962, P = 0.003). HCG positive rates decreased when the dominant follicle was less than 19.0 mm (Group A). Compared to Group A, the likelihood of HCG positive increased by 3.879 times (95% CI 1.296–16.208, P = 0.015) in Group B (19–21.0 mm) and 5.119 times (95% CI 1.674–15.655, P = 0.015) in Group C (>21.0 mm).For unexplained infertility (Table 5), dominant follicle size also emerged as an independent factor influencing HCG positive rate. Patients with follicles in the 17–18.9 mm and 19–21.0 mm ranges were more likely to achieve HCG positive compared to those with follicles larger than 21 mm (adjusted OR 4.282, 95% CI 1.312–13.971, P = 0.016; adjusted OR 3.831, 95% CI 1.298–11.304, P = 0.015, respectively).

Discussion

This study investigated the optimal dominant follicle size on trigger day in patients with ovulatory dysfunction and unexplained infertility undergoing LE-IUI cycles. A retrospective cohort study of 411 matched cycles per group was conducted after 1:1 propensity score matching for couples age, baseline FSH levels, BMI, and other factors influencing IUI pregnancy outcomes. This matching ensured comparable baseline characteristics between the OD and UI groups, thereby facilitating a more robust analysis of the impact of infertility etiology and dominant follicle size on LE-IUI outcomes. Our findings revealed that the optimal dominant follicle size differed between OD and UI patients in LE-IUI cycles. In the OD group,

Table 2 Basic characteristics and clinical outcomes of different dominant follicle size for ovulatory dysfunction patients in LE-IUI cycles ($\bar{x} \pm s$)

	Group A:17–18.9 mm (n = 66)	Group B:19–21.0 mm (n = 177)	Group C: > 21.0 mm (n = 168)	*P* value
Female Age (year)	31.52 ± 3.39	30.84 ± 3.64	31.01 ± 3.24	0.398
Male Age (year)	32.94 ± 4.04	33.28 ± 4.04	33.25 ± 3.71	0.825
Basel FSH (mIU/ml)	7.34 ± 2.18	7.44 ± 2.56	6.91 ± 1.90	0.086
Basel LH (mIU/ml)	8.16 ± 4.21	8.86 ± 5.23	8.54 ± 5.44	0.637
Female BMI (kg/m^2)	20.80 ± 2.46	20.68 ± 2.58	21.04 ± 2.60	0.408
Duration of infertility (years)	3.35 ± 1.52	3.33 ± 1.98	3.73 ± 2.08	0.127
Infertility type, n (%)				0.177
Primary	54 (81.8)	126 (71.2)	118 (70.2)	
Secondary	12 (18.2)	51 (28.8)	50 (28.8)	
Endometrial thickness on trigger day (mm)	9.99 ± 2.01	9.71 ± 2.12	9.44 ± 2.25	0.191
Dominant follicle size (mm)	17.98 ± 0.49	20.08 ± 0.68	23.08 ± 1.45	< 0.001
Number of follicles ≥ 14 mm	1.50 ± 0.93	1.40 ± 0.73	1.53 ± 0.83	0.314
Number of follicles ≥ 16 mm	1.29 ± 0.58	1.32 ± 0.58	1.42 ± 0.65	0.167
Number of follicles ≥ 18 mm	0.79 ± 0.57	1.19 ± 0.44	1.33 ± 0.55	< 0.001
TMCS (*10^6)	116.62 ± 92.39	120.06 ± 92.81	112.68 ± 87.07	0.752
HCG positive rate, n (%)	5 (7.6)	38 (21.5)	44 (26.2)	0.007
Clinical pregnancy rate, n (%)	4 (6.1)	38 (21.5)	43 (25.6)	0.004
Live birth rate, n (%)	3 (4.5)	34 (19.2)	39 (23.2)	0.004
Multiple pregnancy rate, n (%)	0	4 (10.53)	1 (2.33)	0.258
Early miscarriage rate, n (%)	1 (25.0)	3 (8.1)	4 (9.3)	0.549

OD: ouvlatory dysfunction; UI: unexplained infertility; TMCS: the post-preparation total motile count of sperm

P < 0.05 was considered statistically significant

dominant follicles measuring 17–18.9 mm were associated with poorer pregnancy outcomes compared to follicles ≥ 19 mm. Conversely, in the UI group, dominant follicles > 21 mm were associated with a lower positive HCG rate compared to follicles measuring 17–21 mm. Dominant follicle size, therefore, emerged as a significant predictor of positive HCG rate in both OD and UI patients undergoing LE-IUI.

Trigger timing, typically determined by dominant follicle size via ultrasound, is critical because premature or delayed HCG administration can lead to immature oocyte release or follicular atresia, substantially affecting reproductive outcomes [7]. Several previous studies have investigated optimal follicle sizes for LE-IUI cycles, yielding variable findings. Kuang et al. conducted a large retrospective cohort study (n = 3797) analyzing LE + HMG IUI cycles, demonstrating the highest clinical pregnancy rates when dominant follicles measured 16.1–18.0 mm during HCG-triggered cycles. Although the study encompassed patients with diverse infertility etiologies—including ovulatory dysfunction, unexplained infertility, mild male factor infertility, and sexual dysfunction—it lacked subgroup-specific analyses, limiting direct comparative interpretations [4]. A study investigated 939 LE stimulation cycles among women aged ≤ 38 years undergoing artificial insemination by donor (AID) due to male factor infertility. Their findings revealed optimal clinical pregnancy rates when serum LH levels exceeded 10 mIU/mL, estradiol levels were > 300 pg/mL, and dominant follicle sizes were < 19 mm [8]. In contrast, another retrospective analysis of 763 patients undergoing their first LE-IUI cycle for ovulatory dysfunction and unexplained infertility reported better clinical pregnancy rates when follicle sizes ranged between 19.1 and 21.0 mm. Both very large (> 21 mm) and very small (< 16 mm) follicles can diminish success rates [5]. Furthermore, a study involving 1075 women reported higher pregnancy rates with follicles measuring 23–28 mm in both CC and LE cycles, associating optimal size with endometrial thickness. Within that range, pregnancy rates were higher when the larger follicles were accompanied by a thicker endometrium and vice versa [9]. However, these studies varied in their populations, stimulation protocols, and conclusions, and did not specifically analyze infertility causes. Our research addresses these gaps by highlighting differences between infertile populations and providing the first comparative analysis of ideal follicle size in LE-IUI cycles for patients with ovulatory dysfunction versus those with unexplained infertility, thus filling a critical knowledge gap in this field.

Table 3 Basic characteristics and clinical outcomes of different dominant follicle size for unexplained infertility patients in LE-IUI cycles ($\bar{x} \pm s$)

	Group A:17–18.9 mm (n = 83)	Group B:19–21.0 mm (n = 211)	Group C: > 21.0 mm (n = 117)	*P*-value
Female age (year)	30.66 ± 3.52	31.48 ± 3.45	31.06 ± 3.29	0.159
Male age (year)	32.98 ± 4.21	33.72 ± 4.36	33.03 ± 4.31	0.244
Basel FSH (mIU/ml)	7.46 ± 2.04	7.43 ± 1.87	7.16 ± 1.84	0.407
Basel LH (mIU/ml)	4.93 ± 2.48	4.74 ± 2.09	5.05 ± 2.25	0.445
Female BMI (kg/m^2)	21.27 ± 2.80	21.37 ± 2.46	20.83 ± 2.48	0.168
Duration of infertility (years)	3.267 ± 1.64	3.59 ± 1.88	3.25 ± 1.82	0.179
Infertility type, n (%)				
Primary	56 (67.5)	143 (67.8)	86 (73.5)	0.513
Secondary	27 (32.5)	68 (32.2)	31 (26.5)	0.159
Endometrial thickness on trigger day (mm)	9.81 ± 1.87	9.49 ± 2.02	9.42 ± 2.03	0.366
Dominant follicle size (mm)	18.05 ± 0.52	19.91 ± 0.70	22.59 ± 1.05	< 0.001
Number of follicles ≥ 14 mm	1.49 ± 0.756	1.36 ± 0.59	1.44 ± 0.72	0.262
Number of follicles ≥ 16 mm	1.35 ± 0.65	1.23 ± 0.48	1.33 ± 0.59	0.136
Number of follicles ≥ 18 mm	0.90 ± 0.58	1.13 ± 0.36	1.21 ± 0.50	< 0.001
TMCS ($*10^6$)	108.10 ± 84.85	112.00 ± 89.61	123.35 ± 89.64	0.418
HCG positive rate, n (%)	11 (13.3)	25 (11.8)	4 (3.4)	0.023
Clinical pregnancy rate, n (%)	9 (10.8)	22 (10.4)	4 (3.4)	0.065
Live birth rate, n (%)	8 (9.6)	22 (10.4)	4 (3.4)	0.077
Multiple pregnancy rate, n (%)	1 (11.1)	0	0	–
Early miscarriage rate, n (%)	1 (11.1)	0	0	–

OD: vulatory dysfunction; UI: unexplained infertility; TMCS: the post-preparation total motile count of sperm

P < 0.05 was considered statistically significant

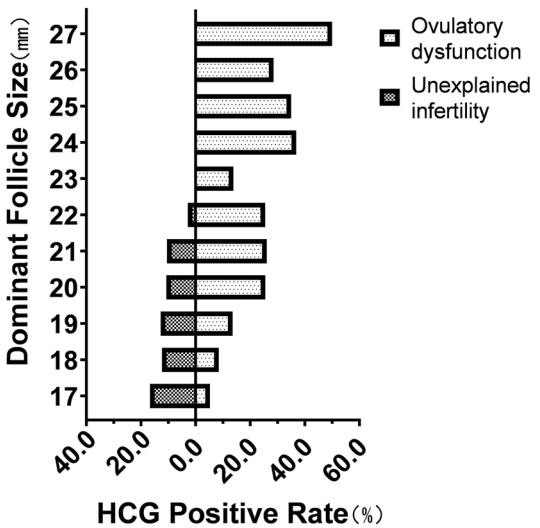

Fig. 1 HCG positive rate of different dominant follicle size of ovulatory dysfunction and unexplained infertility patients

The differences in optimal dominant follicle diameters between the two patient groups may be related to the varying endocrine characteristics of patients with ovulatory dysfunction and unexplained infertility. Some clinicians tend to administer trigger to patients when the LH surge presented, however, as the follicles grow, the likelihood of developing the luteinized unruptured follicle syndrome (LUFS) increases. LUFS is characterized by the failure of mature follicles to rupture and release the oocyte which may hinder pregnancy success. Its incidence significantly increases in ovulation induction cycles, reaching 20–35% [10]. Specifically, in letrozole-induced cycles, LUFS occurrence reaches approximately 18.9%, with a notably higher prevalence when dominant follicle diameter exceeds 22 mm compared to follicles measuring 18–21.9 mm [11]. Furthermore, LUFS prevalence is higher in patients with endometriosis (35% per patient and 25% per cycle) compared to those without (11% per patient and 7% per cycle, P < 0.05) [12]. Endometriosis, a potential cause of unexplained infertility in women aged 25–45, is diagnosed via laparoscopy in up to 90% of unexplained infertility case [13]. In LUFS cycles, with rapid follicular growth and large follicle size, follicular phase FSH and LH levels are lower than in ovulatory cycles. Elevated progesterone, detectable early in the follicular phase, suggests premature endometrial luteinization [14]. Luteinization of the follicles and increased progesterone act on the endometrium,

Table 4 Univariable and multivariable logistic analysis for HCG positive rate in ovulatory dysfunction patients

Variable	Crud OR (CI 95%)	*P* value	Adjust OR (CI 95%)	*P* value
Female age	0.889 (0.827–0.956)	0.001	0.892 (0.828–0.962)	0.003
Male age	0.949 (0.891–1.01)	0.099		
Basel FSH	0.986 (0.886,1.098)	0.801		
Basel LH	1.016 (0.972–1.062)	0.493		
Female BMI (kg/m^2)	0.907 (0.822–1.001)	0.053		
Duration of infertility	0.925 (0.812–1.052)	0.235		
Endometrial thickness on trigger day	1.108 (0.994–1.234)	0.063		
Number of follicles ≥ 14 mm	1.51 (1.158–1.969)	0.002	0.937 (0.465–1.887)	0.855
Number of follicles ≥ 16 mm	1.929 (1.354–2.749)	< 0.001	2.574 (0.908–7.294)	0.075
Number of follicles ≥ 18 mm	1.91 (1.26–2.894)	0.002	0.677 (0.326–1.407)	0.296
Infertility type				
Primary	Ref			
Secondary	1.081 (0.64–1.828)	0.77		
Dominant follicle size group		0.012		0.016
Group A: 17–18.9 mm	Ref			
Group B: 19–21.0 mm	3.335 (1.252–8.885)	0.016	3.879 (1.296–11.608)	0.015
Group C: > 21.0 mm	4.329 (1.634–11.471)	0.003	5.119 (1.674–15.655)	0.004
TMCS	1.002 (0.999–1.004)	0.122		

TMCS: the post-preparation total motile count of sperm

P < 0.05 was considered statistically significant

Table 5 Univariable and multivariable logistic analysis for HCG positive rate in unexplained infertility patients

Variable	Crud OR (CI 95%)	*P* value	Adjust OR (CI 95%)	*P* value
Female Age	0.981 (0.892–1.08)	0.702	0.98(0.89–1.078)	0.674
Male Age	0.99 (0.917–1.069)	0.79		
Basel FSH	1.009 (0.85–1.197)	0.922		
Basel LH	1.128 (0.99–1.285)	0.071		
Female BMI (kg/m^2)	1.094 (0.965–1.24)	0.16		
Duration of infertility	0.991 (0.827–1.188)	0.925		
Endometrial thickness on trigger day	1.069 (0.912–1.253)	0.412		
Number of follicles ≥ 14 mm	1.167 (0.739–1.843)	0.508		
Number of follicles ≥ 16 mm	1.057 (0.591–1.89)	0.852		
Number of follicles ≥ 18 mm	0.976 (0.481–1.98)	0.947		
Infertility type				
Primary	Ref			
Secondary	0.733 (0.347–1.549)	0.416		
Dominant follicle size group		0.037		0.037
Group A: 17–18.9 mm	4.316 (1.324–14.073)	0.015	4.282 (1.312–13.971)	0.016
Group B: 19–21.0 mm	3.797 (1.288–11.193)	0.016	3.831 (1.298–11.304)	0.015
Group C: > 21.0 mm	Ref			
TMCS	1.002 (0.998–1.005)	0.287		

TMCS: the post-preparation total motile count of sperm

P < 0.05 was considered statistically significant

ultimately leading to a decrease in fertilization rate, advancement of the endometrial implantation window, and a poor IUI pregnancy outcome [14]. Notably, no pregnancies occurred in the unexplained infertility group when follicle size exceeded 23 mm in our study. Consequently, in patients with unexplained infertility,

follicles exceeding 21 mm may significantly increase the risk of LUFS and decreased IUI pregnancy rates.

In contrast to unexplained infertility, larger dominant follicles improve LE-IUI pregnancy rates in patients with ovulatory dysfunction. This may be due to gonadotropin surge-attenuating factor (GnSAF). GnSAF is a nonsteroidal ovarian hormone that plays a crucial role in regulating the menstrual cycle by modulating the pituitary response to gonadotropin-releasing hormone (GnRH) [15]. GnSAF is produced by ovarian granulosa cells and acts to reduce the pituitary's responsiveness to GnRH, thereby attenuating the LH surge [16]. The bioactivity of GnSAF is highest in small follicles, particularly those measuring 5–6 mm in diameter [17]. In our study cohort, approximately 90% of patients with ovulatory dysfunction were diagnosed with PCOS, a condition marked by numerous small follicles that contribute to a "polycystic" ovarian appearance. In PCOS patients, large amount of small follicles induce high levels of GnSAF may delay LH surge, preventing follicles from luteinizing and synchronizing the ovulation period with the endometrial implantation window. Thus, even though the dominant follicle diameter is larger, the pregnancy rate in patients with ovulatory dysfunction during the LE-IUI cycle is not adversely affected. Hence, it is advisable to take into account the cause of infertility in patients when administering the trigger during the LE-IUI cycle.

In this current study, we use propensity score matching to control for confounding factors and reduce selection bias, making the data more comparable. This is the first comparative analysis of optimal dominant follicle size in LE-IUI cycles for patients with ovulatory dysfunction versus those with unexplained infertility. Despite these strengths, we also acknowledge some limitations. Firstly, hormonal tests were not routinely conducted on trigger day at our center, making it difficult to determine the impact of follicular luteinization on pregnancy outcomes. Secondly, follicle measurements were performed by different doctors, which could induce potential biases in the data. To minimize inter-observer variability in follicle size measurement, we can adopt three-dimensional (3D) ultrasound assessment or artificial intelligence(AI) automatic measurement of the follicle size in the future. Thirdly, this is a retrospective research, so it only relies on medical record data which can still cause bias. Fourth, our analysis did not differentiate letrozole dosages, which may have influenced the results. Finally, this study's focus on ovulatory dysfunction and unexplained infertility in women under 40 limits the generalizability of its findings to other infertility population.

Conclusion

In conclusion, our retrospective study suggests differing optimal dominant follicle sizes for LE-IUI in women under 40 years old based on infertility etiology. Triggering at $\geq$19.0 mm may be optimal for ovulatory dysfunction, while $\leq$21 mm might improve HCG positive rates in unexplained infertility. These findings underscore the importance of considering underlying infertility causes when determining trigger timing. When determining the trigger for LE-IUI cycles, we recommend that consider not only the dominant follicle size, endometrial thickness, and LH levels but also the patient's infertility factors. This may potentially benefit patients with ovulatory dysfunction and unexplained infertility. However, the retrospective nature of our study and inherent limitations necessitate cautious translation into clinical practice. We emphasize the critical need for prospective, large-scale randomized controlled trials (RCTs) to validate these findings.

Abbreviations

IUI	Intrauterine insemination
LE	Letrozole
OD	Ovulatory dysfunction
UI	Unexplained infertility
OI	Ovulation induction
CC	Clomiphene citrate
PCOS	Polycystic ovary syndrome
TMSC	Total motile sperm count
TVS	Transvaginal ultrasounds
HCG	Human chorionic gonadotropin
LUFS	Luteinized unruptured follicle syndrome
GnSAF	Gonadotropin surge-attenuating factor

Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

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Author contributions

Yihua LIANG was responsible for conducting the research, collecting data, drafting the article, and analyzing the data; Haiyan LIN was responsible for drafting the article, analyzing the data, reviewing the manuscript;Qi QIU, Xuedan JIAO and Ping PAN were responsible for conducting the research and collecting data; Yu LI and Qingxue Zhang were responsible for critically reviewing the intellectual content of the article, securing research funding, and providing guidance.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Sun Yat-sen Memorial Hospital (Approval Number: SYSKY-2023-1054-01) and is registered with China Medical Research Online (Registration Number: MR-44-23-038090S, www.medicalresearch.org.cn).

Human rights statements and informed consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and its later amendments. Informed consent was obtained from all patients for being included in the study.

Competing interests

The authors declare no competing interests.

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