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Ovarian response determines the luteinizing hormone suppression threshold for patients following the gonadotrophin releasing hormone antagonist protocol: A retrospective cohort study

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ABSTRACT

Background: Ovarian reactivity to gonadotrophin stimulation varies, and individual adjustments to the timing and dose of gonadotrophin-releasing hormone (GnRH) antagonist administration are necessary to prevent excessive increases and decreases in luteinizing hormone (LH) levels in patients with different ovarian response following the GnRH antagonist (GnRH-A) protocol. The present study aims to investigate optimal LH suppression thresholds for patients with normal ovarian response (NOR), high ovarian response (HOR), and poor ovarian response (POR) following the GnRH-A protocol respectively.

Methods: A total of 865 in vitro fertilization (IVF) cycles using a flexible or fixed GnRH-A protocol were included. Patients were categorized into the HOR, NOR, or POR group according to their anti-Müllerian hormone (AMH) levels. Then, patients in each group were stratified into one of four subgroups according to the quartile (Q1-Q4) of the basal LH level to LH on triggering day ratio (bLH/hLH). The primary outcomes were the clinical pregnancy and live birth rates, and the secondary outcomes were the number of oocytes retrieved, MII oocytes, two pronucleus (2PN) embryos, and good-quality embryos.

Results: There were 526 patients with NOR, 180 with HOR, and 159 with POR. Basal LH level, LH on triggering day and bLH/hLH were identified as independent predictors of clinical pregnancy rate and live birth rate by logistics regression analysis. Compared to those with NOR, patients with POR had the lowest embryo implantation rate (22.6% vs. 32.8%, $P < 0.05$), clinical pregnancy rate (32.3% vs. 47.3%, $P < 0.05$) and live birth rate (22.6 vs. 37.8%, $P < 0.05$) of fresh embryo transfer (ET). The embryo implantation, clinical pregnancy and live birth rates of frozen embryo transfer (FET) were not significantly different among the three groups. In the subgroup analysis, patients with HOR had the highest embryo implantation rate (51.6%, $P < 0.05$), clinical pregnancy rate (68.4%, $P < 0.05$) and live birth rate (52.6%, $P < 0.05$) of ET in Q3, with a bLH/hLH ratio of 2.40–3.69. In the NOR group, the embryo implantation rate (41.9%, $P < 0.05$), clinical pregnancy rate (61.5%, $P < 0.05$) and live birth rate (50.8%, $P < 0.05$) of ET and live

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birth rate (53.1%, P < 0.05) of FET were highest in Q2, with a bLH/hLH ratio of 1.29–2.05. Patients with POR had the highest clinical pregnancy rate (57.1%, P < 0.05) and live birth rate (42.9%, P < 0.05) of ET in Q2, with a bLH/hLH ratio of 0.86–1.35.
Conclusions: In the present study, the bLH/hLH ratio represented the LH suppression threshold. The subgroup analysis of HOR, NOR and POR showed that, the LH suppression threshold varies according to ovarian response. We recommend LH suppression thresholds of 2.40–3.69 for HOR, 1.29–2.05 for NOR, and 0.86–1.35 for POR to obtain the highest clinical pregnancy rate and live birth rate. This study provides comprehensive and precise references for clinicians to monitor LH levels individually during controlled ovarian stimulation (COS) according to the patient's ovarian response following the GnRH-A protocol.

1. Introduction

In vitro fertilization (IVF) is an important method for the treatment of infertility, which afflicts approximately 15% of families worldwide [1]. Optimizing and individualizing controlled ovarian stimulation (COS) is critical for enhancing the success of in vitro fertilization [2,3]. The gonadotrophin-releasing hormone antagonist (GnRH-A) protocol has gained popularity as a mainstream clinical program for IVF patients in recent years [4–7], as it prevents an untimely luteinizing hormone (LH) surge and premature luteinization.

However, the secretion and response of LH levels to gonadotrophin-releasing hormone (GnRH) antagonists can vary significantly between individuals [8,9]. During the follicular development stage, LH levels can impact the morphological and functional aspects of follicles, determining follicular meiotic status and fertilization ability [10]. Therefore, monitoring LH levels during COS is essential to prevent unpredictable oversuppression of endogenous LH levels and its negative impact on follicle development in clinical practice [11,12]. Previous studies also found that LH levels that are too high or too low can have adverse effects on oocytes, embryo quality and clinical pregnancy outcomes [13–17]. The GnRH-A protocol is a convenient treatment option with few side effects, and can reduce the long recovery period of the downregulating effect, and can reduce the occurrence of ovarian hyperstimulation syndrome (OHSS). Hence, the GnRH-A protocol is suitable for patients with different ovarian responses [18,19].

Anti-Müllerian hormone (AMH) is a glycoprotein hormone produced by the granulosa cells of preantral and small antral follicles [20,21]. AMH levels have been shown to be to positively correlated with the size of the primordial follicle pool and the number of antral follicles [22,23]. Randomized controlled trials have suggested that AMH performs better than the antral follicle count (AFC) in predicting both poor and high ovarian response [24,25]. In the current study, ovarian response was categorized into three groups according to AMH levels: poor ovarian response (POR); normal ovarian response (NOR); and high ovarian response (HOR). For POR, at least two of the following three features must be present: (i) advanced maternal age (≥40 years) or any other risk factor for POR; (ii) a previous POR (≤3 oocytes with a conventional stimulation protocol); or (iii) an abnormal ovarian reserve test (AFC < 5–7 follicles or AMH < 0.5 −1.1 ng/ml) [26,27]. In contrast, a high ovarian response indicates that the ovary is extremely sensitive to gonadotrophin stimulation, with an AMH level >4.5 ng/ml [28], which is a significant factor that may lead to OHSS [29,30]. Ovarian reactivity to gonadotrophin stimulation is different. Hence, the timing and dose of GnRH antagonist administration should be adjusted accordingly to avoid excessive increases or decreases in LH levels, depending on the ovarian response of patients during the GnRH-A protocol. Previous studies have shown that low basal LH levels or low LH levels on triggering day have a negative impact on the clinical pregnancy rates of patients following the GnRH-A protocol [31–33]. However, there has been no research focusing on the effects of LH suppression levels on reproductive outcomes in HOR, NOR and POR patients following the GnRH-A protocol respectively.

The ratio of basal LH to LH on triggering day (bLH/hLH) can indicate LH suppression levels during COS for IVF patients who follow the GnRH-A protocol. This study aims to investigate the relationship between bLH/hLH ratios and reproductive outcomes in different ovarian response IVF patients following the GnRH-A protocol. Thereafter, the optimal LH suppression threshold will been provided for different ovarian response patients following the GnRH-A protocol respectively.

2. Methods

2.1. Study population and design

This was a retrospective study including 865 cycles from January 2021 to January 2022 at our reproductive medicine centre. The inclusion criteria were as follows: (1) patients undergoing in vitro fertilization (IVF) following the GnRH-A protocol and (2) patients who underwent blood testing for measurements of basal LH and LH levels on triggering day between 8 and 9 am. The exclusion criteria for the patients were as follows: (1) endometriosis or adenomyosis; (2) autoimmune, endocrine, or metabolic disease; (3) uterine abnormalities; (4) uterine adhesion; (5) chromosomal abnormalities; (6) recurrent spontaneous abortion; (7) use of donor sperm or ovum; or (8) use of oral contraceptives before COS.

2.2. Group setting

According to serum AMH levels, patients were divided into the "high ovarian response" (HOR) group, with AMH >4.5 ng/ml [28], the "normal ovarian response" (NOR) group, with AMH 1.1–4.5 ng/ml, and the "poor ovarian response" (POR) group, with these

patients having at least two of the following three features: (i) advanced maternal age (≥40 years) or any other risk factor for POR; (ii) a previous POR (≤3 oocytes with a conventional stimulation protocol); or (iii) an abnormal ovarian reserve test (AFC < 5–7 follicles or AMH < 0.5−1.1 ng/ml) [26,27]. To the effect of the bLH/hLH ratio on reproductive outcomes in patients with according to the ovarian response classification, we divided patients in each group into four subgroups according to the quartile of the bLH/hLH ratio. The subgroups were Q1 (<1.49), Q2 (1.49–2.40), Q3 (2.40–3.69), and Q4 (>3.69) in the HOR group; Q1 (<1.29), Q2 (1.29–2.05), Q3 (2.05–3.20), and Q4 (>3.20) in the NOR group; and Q1 (<0.86), Q2 (0.86–1.35), Q3 (1.35–2.32), and Q4 (>2.32) in the POR group. A flowchart of the study population is presented in Fig. 1.

2.3. Controlled ovarian stimulation

The initial dose of gonadotropin was determined according to age, AMH, antral follicle count (AFC), and basal endocrine hormone levels. The starting dose of gonadotropin (Gn) ranged from 150 to 375 IU. Follicle development was detected by transvaginal ultrasonography, and the gonadotropin dosage was adjusted according to the ovarian response of the patient, E_2 level, and number of follicles. When one of following criteria was met: (1) presence of at least one follicle ≥14 mm, (2) E_2 level ≥600 pg/ml, or (3) LH level ≥10 IU/L [34], GnRH-A was administered daily from day of COS in fixed protocol and was initiated in flexible protocol.

When at least two follicles reached a mean diameter of >17 mm or when one follicle reached a mean diameter of 18 mm, final oocyte maturation was triggered. HCG or GnRH agonist was used alone or in combination in the GnRH-A protocol. Oocyte retrieval was performed 34–38 h after triggering by transvaginal ultrasound-guided aspiration. Then, a standard IVF procedure was followed as indicated.

2.4. Sperm retrieval

After semen was obtained by masturbation and collected in a sterile and nontoxic container, the container was placed on a shaking worktable at room temperature until the semen was completely liquefied. Then, the semen was processed to reduce its viscosity, promote sperm capacitation and improve the sperm's fertilizing ability.

2.5. In vitro fertilization and embryo transfer

After oocyte retrieval, oocytes were maintained in culture medium for several hours, and the processed sperm was added to the culture medium. After 16–18 h, the oocytes were observed to determine whether they were fertilized. A primary pronucleus embryo was defined as a fertilized oocyte with an intact zona pellucida, healthy cytoplasm, and two clear pronuclei. Fresh embryo transfer (ET) was usually performed on days 2–3 after fertilization with a zygote, defined as a cleavage-stage embryo, or on days 5–6 after fertilization with a blastocyst. Frozen embryo transfer (FET) was performed with a similar surgical procedure to that of fresh ET. Embryo transplantation was initiated only when embryo development and endometrial preparation were synchronized to ensure endometrial receptivity of the embryo. Embryo transfer was performed with ultrasound guidance. No more than two embryos were transferred. All patients received luteal phase support.

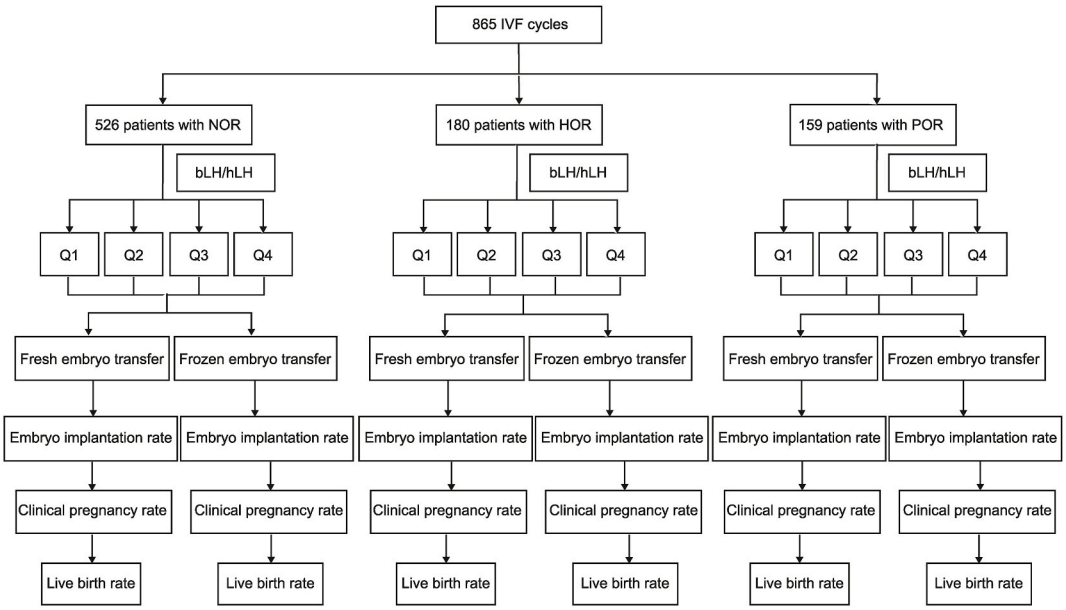

Fig. 1. Flowchart of the study population.

2.6. Embryo grading

For cleavage-stage embryos, good-quality embryos were defined as those with at least 4 cells 48 h after oocyte retrieval or those with 6 cells classified as grade 2 embryos 72 h after oocyte retrieval [35]. Blastocyst grading was based on the expansion of the blastocyst cavity, the development of the inner cell mass, and the development of trophoblast cells. In the evaluation of expansion of the blastocyst cavity, expansion was graded as follows: grade 1, blastocyst cavity volume below half of the total blastocyst volume; grade 2, blastocyst cavity volume above half of the total blastocyst volume; grade 3, blastocyst cavity volume equal to blastocyst volume; grade 4, blastocyst cavity exceeding size of the blastocyst and zona pellucida that is thinner; grade 5, hatching blastocyst that is in the process of detaching from the zona pellucida; and grade 6, detachment of the entire blastocyst from the zona pellucida. In the evaluation of the development of the inner cell mass, which can develop into an embryo, inner cell mass was graded as follows: grade A, many cells that are closely arranged; grade B, fewer cells than grade A and loose arrangement; and grade C, few cells. In the evaluation of the development of trophoblast cells, which are peripheral cells that absorb nutrients in the uterine cavity, trophoblast cells were graded as follows: grade A, having a dense epithelial cell layer; grade B, having a loosely structured epithelial cell layer; and grade C, having few cells with an epithelial cell layer structure that is more sparsely that of the grade B.

2.7. Embryos freezing and thawing

For embryo vitrification, preparation of the operation dish containing the base solution and the balance solution at 37 °C was prepared, and the embryos were transferred to the middle position above the droplet of the balance solution, allowing them to slowly sink. Then, the embryos were transferred to at least 3 to 5 different positions in the freezing solution to remove any residual balance solution. Finally, they were loaded and stored in liquid nitrogen. For embryo thawing after vitrification, the embryo was quickly removed from liquid nitrogen (within 1 s) and immersed in thawing solution for 45–60 s. The sample floating on the upper layer of the thawing solution was moved to the centre of the droplet. Then, the sample was transferred to the diluent and incubated for 3 min. Next, the sample was transferred to the base solution and incubated for 5 min. Finally, the sample was transferred to a new base solution and incubated for 5 min. For embryo slow cooling, the embryo was placed into a straw, and the straw was placed into the freezing device. The freezing program was as follows: starting temperature of 20 °C, −2 °C/min from 20 °C to −7 °C, −7 °C for 5 min, −7 °C for 10 min while planting the ice crystal, −0.3 °C/min from −7 °C to −30 °C, and then −30 °C/min from −30 °C to −150 °C. For embryo thawing for slow cooling, the thawing solutions T1, T2, T3, and T4 were prepared. The straw was removed from the liquid nitrogen and kept in the air for 40 s. The straw then was soaked in a 30 °C water bath for 40 s, and the straw was dried. The embryo was blown into T1 and kept there for 5 min. Then, the embryo was transferred to T2 and kept there for 5 min, followed by transfer to T3 and incubation there for 10 min. Finally, the embryo was transferred to T4 and kept there for 10 min. When thawing process was complete, the embryo could be cultured.

2.8. Reproductive outcomes

The primary outcomes were the clinical pregnancy rate and live birth rate. The embryo implantation rate was defined as the ratio of the number of gestational sacs observed by ultrasound to the total number of embryos transferred. Clinical pregnancy was defined as a pregnancy diagnosed by ultrasonographic visualization of one or more gestational sacs. Live birth was defined as the birth of an infant with evidence of life after 28 weeks of gestational age. This study were oocytes retrieved; MII oocytes; MII oocyte rate, defined as the ratio of MII oocytes to oocytes retrieved; 2PN embryos, defined as fertilized oocytes with two-pronuclei; 2PN embryo rate, defined as the ratio of 2PN embryos to oocytes retrieved; good-quality embryos, defined as at least 4 cells 48 h after oocyte retrieval or embryos with 6 cells that were classified as grade 2 embryos 72 h after oocyte retrieval [35], and good-quality embryo rate, defined as the ratio of good-quality embryos to oocytes retrieved.

2.9. Statistical analysis

All analyses were performed using SPSS Statistics 21. The Kolmogorov–Smirnov test was used to determine whether the variables were distributed normally. If the variables were normally distributed, one-way ANOVA was used. If the variables were nonnormally distributed, the Kruskal–Wallis test was used. The variables with a normal distribution are expressed as the mean ± SD, and categorical variables were presented as frequencies and percentages. Categorical variables were compared using the chi-squared test, and if any cell in the chi-square test had an expected frequency less than 5, Fisher's exact test was used. Multiple linear regression or logistic regression analyses were used to control certain confounders among groups. All statistical tests were two sided, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Clinical characteristics and reproductive outcomes in different groups

A total of 865 women who used the GnRH-A protocol as the COS protocol were included in the present study. All variables were initially included in the logistic regression. The results showed that the basal LH level, LH on triggering day and bLH/hLH ratio were independent predictors of the clinical pregnancy rate (Fig. 2A) and live birth rate of ET (Fig. 2B). Due to differences in LH levels among

patients with differing ovarian responses, the patients were divided into three groups based on their serum AMH levels for further analyses: HOR, NOR and POR. There were 526 patients with NOR, 180 patients with HOR, and 159 patients with POR. Compared with patients with NOR, those with HOR were younger and had a longer menstrual cycle, a higher AFC, a higher basal LH level, a higher AMH level, a higher bLH/hLH ratio, and higher E_2 and P levels on triggering day, but lower basal FSH and E_2 levels. Patients with POR were older than those with NOR and had higher basal FSH levels, lower basal LH and P levels, a lower AFC, lower AMH levels, a lower bLH/hLH ratio, and lower E_2 and P levels on triggering day. When compared to those with HOR, patients with POR were older and had higher basal FSH levels, higher LH on triggering day, a shorter menstrual cycle, lower basal LH levels, a lower AFC, lower AMH levels, a lower bLH/hLH ratio, and lower E_2 and P levels on triggering day. Compared with that used in patient with NOR and POR, less Gn was used in patients with HOR. Details regarding the baseline clinical characteristics of the participants were shown in Table 1.

During COS, more oocytes retrieved (15.74 ± 6.04 vs. 9.37 ± 5.65, $p < 0.001$), MII oocytes (14.75 ± 5.98 vs. 8.69 ± 5.20, $p < 0.001$), 2PN embryos (9.98 ± 5.39 vs. 5.70 ± 4.17, $p < 0.001$) and good-quality embryos (3.91 ± 3.34 vs. 2.35 ± 2.30, $p < 0.001$) were observed in HOR patients. Although patients with HOR had a lower good-quality embryo rate (0.25 ± 0.18 vs. 0.28 ± 0.25) than those with NOR, no significant differences were observed. Fewer oocytes retrieved (3.53 ± 2.32 vs. 9.37 ± 5.65, $p < 0.001$), MII oocytes (3.31 ± 2.21 vs. 8.69 ± 5.20, $p < 0.001$), 2PN embryos (2.27 ± 1.82 vs. 5.70 ± 4.17, $p < 0.001$) and good-quality embryos (1.30 ± 1.35 vs. 2.35 ± 2.30, $p < 0.001$) were observed in POR patients. However, patients with POR had a higher good-quality embryo rate (0.37 ± 0.35 vs. 0.28 ± 0.25, $p < 0.05$) than those with NOR. When compared with HOR patients, POR patients had fewer oocytes retrieved (3.53 ± 2.32 vs. 15.74 ± 6.04, $p < 0.001$), MII oocytes (3.31 ± 2.21 vs. 14.75 ± 5.98, $p < 0.001$), MII oocytes (0.91 ± 0.22 vs. 0.93 ± 0.11, $p < 0.05$), 2PN embryos (2.27 ± 1.82 vs. 9.98 ± 5.39, $p < 0.001$) and good-quality embryos (1.30 ± 1.35 vs. 3.91 ± 3.34, $p < 0.001$). Patients with POR had a higher good-quality embryo rate (0.37 ± 0.35 vs. 0.25 ± 0.18, $p < 0.05$) than those with HOR.

The ET rate in the HOR group was lower than that in the NOR group (47.8% vs. 55.9%, $p < 0.05$), but no significant difference was found in the embryo implantation rate, clinical pregnancy rate or live birth rate of ET between the HOR and NOR groups. In comparison to that in the NOR group, the ET rate of the POR group was lower (39.0% vs. 55.9%, $p < 0.05$). Although the good-quality embryo rate was higher among POR patients than among NOR patients, the embryo implantation rate (22.6% vs. 32.8%, $p < 0.05$), clinical pregnancy rate (32.3% vs. 47.3%, $p < 0.05$) and live birth rate (22.6% vs. 37.8%, $p < 0.05$) of ET were lower among patients with POR than among those with NOR. Compared with those of the HOR group, the ET, embryo implantation, clinical pregnancy and live birth rates of the POR group were not significantly different. There was a significant difference in the luteal phase support schemes of ET among the three groups. The FET rate was higher in the HOR group than in the NOR (43.3% vs. 28.1%, $p < 0.001$) and POR (43.3% vs. 24.5%, $p < 0.001$) groups. Only one POR cycle was performed with fresh blastocyst transfer. The blastocyst implantation rate and nonblastocyst implantation rate of FET were not significantly different among the three groups. The luteal phase support schemes of FET and the embryo implantation, clinical pregnancy and live birth rates were not significantly different among the

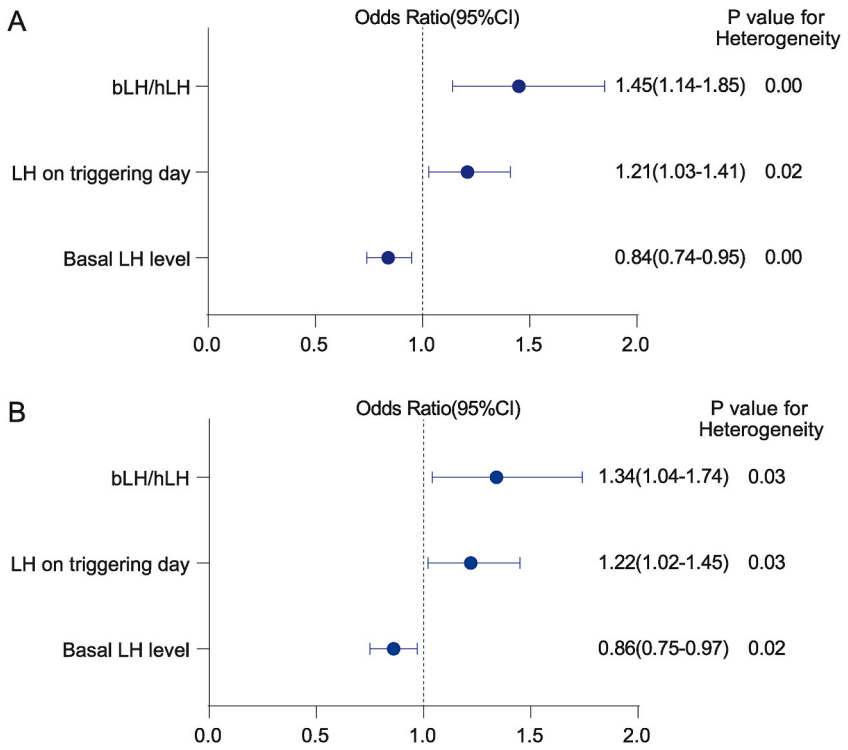

Fig. 2. Logistic regression analysis of clinical pregnancy rate (A) and live birth rate (B) of fresh embryo transfer.

Table 1
Baseline characteristics and reproductive outcomes in NOR, HOR and POR.

Characteristics	NOR (526)	HOR (180)	P_a	POR (159)	P_b	P_c
Maternal age (y)	31.96 ± 4.16	29.36 ± 3.68	**<0.001**	34.15 ± 4.86	**<0.001**	**<0.001**
Maternal BMI (kg/m^2)	21.42 ± 2.73	21.75 ± 2.69	0.16	21.18 ± 2.75	0.35	0.06
Menstrual cycle	31.94 ± 12.18	38.77 ± 20.17	**<0.001**	29.93 ± 9.54	0.06	**<0.001**
AFC	5.10 ± 2.40	6.65 ± 2.86	**<0.001**	2.92 ± 1.51	**<0.001**	**<0.001**
Basal FSH (IU/L)	7.01 ± 2.05	6.18 ± 1.54	**<0.001**	9.50 ± 3.87	**<0.001**	**<0.001**
Basal LH (IU/L)	5.62 ± 3.00	6.66 ± 3.40	**<0.001**	4.97 ± 2.10	**<0.05**	**<0.001**
Basal E_2 (pmol/L)	117.92 ± 40.59	110.72 ± 37.06	**<0.05**	110.62 ± 42.33	0.07	0.98
Basal P (nmol/L)	1.24 ± 0.55	1.19 ± 0.52	0.38	1.13 ± 0.52	**<0.05**	0.29
T (nmol/L)	0.87 ± 0.74	0.93 ± 0.48	0.46	0.91 ± 0.71	0.65	0.07
AMH (ng/ml)	3.13 ± 2.90	6.42 ± 1.42	**<0.001**	0.67 ± 0.28	**<0.001**	**<0.001**
LH on triggering day (IU/L)	3.17 ± 2.25	3.19 ± 2.31	0.93	4.11 ± 2.92	**<0.001**	**<0.05**
E_2 on triggering day (pmol/L)	9876.49 ± 6439.62	15213.73 ± 8415.11	**<0.001**	4146.94 ± 2472.19	**<0.001**	**<0.001**
P on triggering day (nmol/L)	2.70 ± 2.21	3.10 ± 1.76	**<0.05**	1.67 ± 0.85	**<0.001**	**<0.001**
Total Gn (IU)	2116.52 ± 625.46	1796.24 ± 674.53	**<0.001**	2220.00 ± 715.67	0.08	**<0.001**
bLH/hLH	2.42 ± 1.55	2.83 ± 1.73	**<0.001**	1.77 ± 1.29	**<0.001**	**<0.001**
Trigger schemes			**<0.001**		0.09	**<0.05**
HCG	489(93.0%)	155(86.1%)		154(96.9%)		
Dual trigger	22(4.2%)	10(5.6%)		4(2.5%)		
GnRH-a	15(2.9%)	15(8.3%)		1(0.6%)		
Oocytes retrieved	9.37 ± 5.65	15.74 ± 6.04	**<0.001**	3.53 ± 2.32	**<0.001**	**<0.001**
MII oocytes	8.69 ± 5.20	14.75 ± 5.98	**<0.001**	3.31 ± 2.21	**<0.001**	**<0.001**
MII oocyte rate	0.94 ± 0.13	0.93 ± 0.11	0.74	0.91 ± 0.22	0.13	**<0.05**
2PN embryos	5.70 ± 4.17	9.98 ± 5.39	**<0.001**	2.27 ± 1.82	**<0.001**	**<0.001**
2PN embryo rate	0.62 ± 0.27	0.63 ± 0.25	0.72	0.63 ± 0.34	0.31	0.41
Good-quality embryos	2.35 ± 2.30	3.91 ± 3.34	**<0.001**	1.30 ± 1.35	**<0.001**	**<0.001**
Good-quality embryo rate	0.28 ± 0.25	0.25 ± 0.18	0.67	0.37 ± 0.35	**<0.05**	**<0.05**
Fresh embryo transfer rate	294(55.9%)	86(47.8%)	**<0.05**	62(39.0%)	**<0.001**	0.10
Luteal phase support schemes			**<0.05**		**<0.001**	**<0.001**
Luteal phase support with E_2	209(71.1%)	50(58.1%)		59(95.2%)		
Luteal phase support without E_2	85(28.9%)	36(41.9%)		3(4.8%)		
Embryo implantation rate	173/528(32.8%)	50/153(32.7%)	0.98	24/106(22.6%)	**<0.05**	0.08
Clinical pregnancy rate	139(47.3%)	40(46.5%)	0.90	20(32.3%)	**<0.05**	0.08
Live birth rate	111(37.8%)	31(36.0%)	0.77	14(22.6%)	**<0.05**	0.08
Frozen embryo transfer rate	148(28.1%)	78(43.3%)	**<0.001**	39(24.5%)	0.37	**<0.001**
Embryo implantation rate	86/245(35.1%)	57/128(44.5%)	0.08	21/63(33.3%)	0.79	0.14
blastocyst implantation rate	19/34(55.9%)	18/34(52.9%)		1/2(50%)		
Non-blastocyst implantation rate	67/211(31.8%)	39/94(41.5%)		20/61(32.8%)		
Luteal phase support schemes			0.74		0.08	0.06
Luteal phase support with E_2	124(83.8%)	64(82.1%)		37(94.9%)		
Luteal phase support without E_2	24(16.2%)	14(17.9%)		2(5.1%)		
Clinical pregnancy rate	71(48.0%)	45(57.7%)	0.17	18(46.2%)	0.84	0.24
Live birth rate	59(39.9%)	41(52.6%)	0.07	15(38.5%)	0.87	0.15

Note: continuous data are presented as mean ± SD and categoric data are presented as n (%). The P_a value is the value for HOR compared to the NOR, P_b value is the value for POR compared to the NOR, and P_c value is the value for HOR compared to the POR. BMI: Body mass index; AFC: Antral follicle count; GnRH-a: Gonadotropin-releasing hormone agonist; Gn: Gonadotropin; FSH: Follicle stimulating hormone; P: Progesterone; LH: Luteinizing hormone; E_2: Estradiol; T: Testosterone; AMH: Anti-Mullerian hormone; HCG: Human chorionic gonadotropin; bLH: Basal LH; hLH: LH on triggering day.

three groups. Details of the comparisons of all three groups are presented in Table 1.

3.2. Reproductive outcomes of patients with NOR

The 526 patients with NOR were divided into four groups (Q1-Q4) according to the quartile of the bLH/hLH ratio, and the baseline characteristics of NOR are shown in Supplemental Table 1. The number of oocytes retrieved increased from 7.30 ± 4.57 in Q1 to 11.87 ± 6.81 in Q4 ($P < 0.01$), and the number of MII oocytes increased from 6.76 ± 4.25 in Q1 to 10.87 ± 6.07 in Q4 ($P < 0.01$). The number of 2PN embryos increased from 4.50 ± 3.64 in Q1 to 6.91 ± 4.87 in Q4 ($P < 0.05$). The number of good-quality embryos increased from 1.86 ± 1.81 in Q1 to 3.03 ± 2.77 in Q4 ($P < 0.05$). No significant differences in the MII oocyte rate and 2PN embryo rate were observed. The good-quality embryo rate was higher in Q4. The embryo implantation rate (41.9%, $P < 0.05$), clinical pregnancy rate (61.5%, $P < 0.05$) and live birth rate (50.8%, $P < 0.05$) of ET were highest in Q2. The embryo implantation rate (42.9%) and clinical pregnancy rate (59.4%) of FET were highest in Q2, although the difference was not significant. The live birth rate (53.1%, $P < 0.05$) of FET was also the highest in Q2. These results indicated that patients in Q2 with a bLH/hLH ratio of 1.29–2.05 had the highest embryo implantation, clinical pregnancy and live birth rates (Table 2, Fig. 3A and B and Fig. 4A and B).

3.3. Reproductive outcomes of patients with HOR

The 180 patients in the HOR group were divided into four groups (Q1-Q4) based on the quartile of the bLH/hLH ratio, and their baseline characteristics were shown in Supplemental Table 2. The number of oocytes retrieved increased from 14.13 ± 6.92 in Q1 to 17.16 ± 5.53 in Q4 ($P < 0.05$), and the number of MII oocytes increased from 12.93 ± 6.63 in Q1 to 16.33 ± 5.27 in Q4 ($P < 0.05$). The number of 2PN embryos increased from 8.27 ± 5.35 in Q1 to 11.20 ± 5.18 in Q4 ($P < 0.05$). The number of good-quality embryos increased from 2.58 ± 2.68 in Q1 to 4.62 ± 3.94 in Q4 ($P < 0.05$). There were no significant differences in the MII oocyte rate, 2PN embryo rate or good-quality embryo rate. The embryo implantation rate (51.6%), clinical pregnancy rate (68.4%) and live birth rate (52.6%) of ET were highest in Q3 ($P < 0.05$). The embryo implantation rate (54.1%), clinical pregnancy rate (68.2%) and live birth rate (63.6%) of FET were highest in Q3, but the difference was not significant. These results indicated that patients in Q3 with a bLH/hLH ratio of 2.40–3.69 had higher rates of embryo implantation, clinical pregnancy and live birth (Table 3, Fig. 3A and B and Fig. 4A and B).

3.4. Reproductive outcomes of patients with POR

Similarly, the 159 patients in the POR group were also separated into four groups (Q1-Q4) according to the quartile of the bLH/hLH ratio, and their baseline characteristics were presented in Supplemental Table 3. The number of oocytes retrieved, MI oocytes, 2PN embryos and good-quality embryos were higher in Q2 and Q3 ($P < 0.05$). The good-quality embryo rate was highest in Q2, but no significant differences were observed. Furthermore, the clinical pregnancy rate (57.1%, $P < 0.05$) and live birth rate (42.9%, $P < 0.05$) of ET were highest in Q2. The embryo implantation rate (34.8%) of ET was also highest in Q2, but there was no significant difference among the four groups. The embryo implantation rate (46.7%), clinical pregnancy rate (60.0%) and live birth rate (50.0%) of FET were highest in Q2; however, there were also no significant differences among the four groups. These results implied that patients in Q2 with a bLH/hLH ratio of 0.86–1.35 had the highest embryo implantation rate, clinical pregnancy rate and live birth rate (Table 4, Fig. 3A and B and Fig. 4A and B).

4. Discussion

In the present study, the patients were categorized into three groups based on their AMH levels: HOR, NOR, and POR. Subgroup analysis in the three groups respectively revealed that the bLH/hLH ratio was associated with reproductive outcomes in patients with different ovarian responses. Specifically, in the HOR group, patients with a bLH/hLH ratio of 2.40–3.69 had the highest embryo implantation rate (51.6%, $P < 0.05$), clinical pregnancy rate (68.4%, $P < 0.05$) and live birth rate (52.6%, $P < 0.05$) of ET and the highest embryo implantation rate (54.1%), clinical pregnancy rate (68.2%) and live birth rate (63.6%) of FET. In the NOR group, patients with a bLH/hLH ratio of 1.29–2.05 had the highest embryo implantation rate (41.9%, $P < 0.05$), clinical pregnancy rate (61.5%, $P < 0.05$) and live birth rate (50.8%, $P < 0.05$) of ET and the highest embryo implantation rate (42.9%), clinical pregnancy rate (59.4%) and live birth rate (53.1%, $P < 0.05$) of FET. Among POR patients, those with a bLH/hLH ratio of 0.86–1.35 had the highest embryo implantation rate (34.8%), clinical pregnancy rate (57.1%, $P < 0.05$) and live birth rate (42.9%, $P < 0.05$) of ET and the highest embryo implantation rate (46.7%), clinical pregnancy rate (60.0%) and live birth rate (50.0%) of FET. The AMH level is easily obtainable in clinical practice and can be used to preliminarily classify patients' ovarian response before the start of COS. During ovarian stimulation, the dosage of GnRH antagonist can be adjusted based on the patient's ovarian response and basal LH level, along with the LH suppression threshold provided in this study. We recommend LH suppression thresholds of 2.40–3.69 for HOR, 1.29–2.05

Table 2
Reproductive outcomes for patients with NOR.

	Ratio of basal LH to LH on triggering day (bLH/hLH)				P value	
Outcomes	Q1(132) (< 1.29)	Q2(132) (1.29–2.05)	Q3(131) (2.05–3.20)	Q4(131) (>3.20)	P^a	P^b
Oocytes retrieved	7.30 ± 4.57	8.26 ± 4.41	10.08 ± 5.43	11.87 ± 6.81	**<0.001**	**<0.001**
MII oocytes	6.76 ± 4.25	7.75 ± 4.20	9.40 ± 5.15	10.87 ± 6.07	**<0.001**	**<0.001**
MII oocyte rate	0.93 ± 0.15	0.94 ± 0.11	0.94 ± 0.11	0.93 ± 0.13	0.77	0.21
2PN embryos	4.50 ± 3.64	5.19 ± 3.46	6.22 ± 4.21	6.91 ± 4.87	**<0.001**	**<0.001**
2PN embryo rate	0.62 ± 0.29	0.63 ± 0.26	0.63 ± 0.27	0.61 ± 0.28	0.94	0.22
Good-quality embryos	1.86 ± 1.81	2.18 ± 2.12	2.32 ± 2.25	3.03 ± 2.77	**<0.001**	**<0.001**
Good-quality embryo rate	0.29 ± 0.27	0.28 ± 0.25	0.25 ± 0.22	0.30 ± 0.26	0.43	**<0.001**
Fresh embryo transfer rate	85(64.4%)	65(49.2%)	81(61.8%)	63(48.1%)	**<0.05**	**<0.05**
Embryo implantation rate	42/146(28.8%)	52/124(41.9%)	49/146(33.6%)	30/112(26.8%)	0.05	**<0.05**
Clinical pregnancy rate	35(41.2%)	40(61.5%)	40(49.4%)	24(38.1%)	**<0.05**	**<0.05**
Live birth rate	26(30.6%)	33(50.8%)	31(38.3%)	20(31.7%)	0.06	**<0.05**
Frozen embryo transfer rate	29(22.0%)	32(24.2%)	34(26.0%)	53(40.5%)	**<0.05**	**<0.05**
Embryo implantation rate	13/45(28.9%)	24/56(42.9%)	23/58(39.7%)	26/86(30.2%)	0.30	0.05
Clinical pregnancy rate	12(41.4%)	19(59.4%)	20(58.8%)	20(37.7%)	0.11	0.05
Live birth rate	9(31.0%)	17(53.1%)	16(47.1%)	17(32.1%)	0.15	**<0.05**

Note: continuous data are presented as mean ± SD and categoric data are presented as n (%). The P value is the value for subgroups comparison and P value with bold text means $P < 0.05$. P^a: uncontrolled for any indicators. P^b: controlled for maternal age, maternal BMI, AMH, basal P level, basal LH level, LH, P and E_2 level on the triggering day, oocytes retrieved, MII oocytes, 2PN embryos and good-quality embryos.

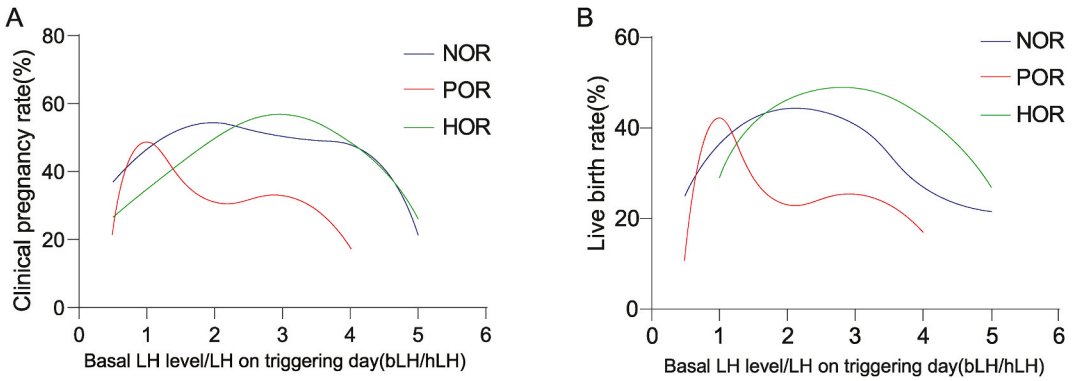

Fig. 3. The fitted graph of clinical pregnancy rate (A) and live birth rate (B) of fresh embryo transfer (ET) as a function of bLH/hLH variation.

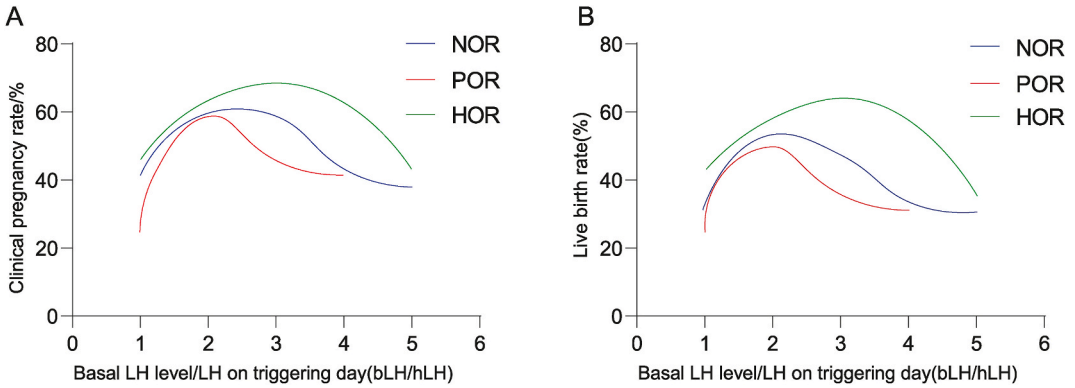

Fig. 4. The fitted graph of clinical pregnancy rate (A) and live birth rate (B) of frozen embryo transfer (FET) as a function of bLH/hLH variation.

Table 3
Reproductive outcomes for patients with HOR.

	Ratio of basal LH to LH on triggering day (bLH/hLH)				P value	
Outcomes	Q1(45) (<1.49)	Q2(45) (1.49–2.40)	Q3(45) (2.40–3.69)	Q4(45) (>3.69)	P^a	P^b
Oocytes retrieved	14.13 ± 6.92	14.64 ± 5.94	17.04 ± 5.17	17.16 ± 5.53	**<0.05**	**<0.001**
MII oocytes	12.93 ± 6.63	13.89 ± 6.05	15.84 ± 5.39	16.33 ± 5.27	**<0.05**	**<0.001**
MII oocyte rate	0.91 ± 0.14	0.94 ± 0.10	0.93 ± 0.13	0.96 ± 0.07	0.52	0.69
2PN embryos	8.27 ± 5.35	9.76 ± 5.89	10.71 ± 4.81	11.20 ± 5.18	0.05	**<0.001**
2PN embryo rate	0.59 ± 0.25	0.64 ± 0.30	0.64 ± 0.22	0.66 ± 0.24	0.58	0.68
Good-quality embryos	2.58 ± 2.68	4.24 ± 3.31	4.20 ± 3.03	4.62 ± 3.94	**<0.05**	**<0.05**
Good-quality embryo rate	0.20 ± 0.15	0.29 ± 0.22	0.24 ± 0.16	0.26 ± 0.18	0.12	0.23
Fresh embryo transfer rate	28(62.2%)	21(46.7%)	19(42.2%)	18(40.0%)	0.14	0.99
Embryo implantation rate	11/49(22.4%)	15/39(38.5%)	16/31(51.6%)	8/34(23.5%)	**<0.05**	**<0.05**
Clinical pregnancy rate	9(32.1%)	12(57.1%)	13(68.4%)	6(33.3%)	**<0.05**	**<0.05**
Live birth rate	5(17.9%)	10(47.6%)	10(52.6%)	6(33.3%)	0.06	**<0.05**
Frozen embryo transfer rate	14(31.1%)	19(42.2%)	22(48.9%)	23(51.1%)	0.22	0.53
Embryo implantation rate	11/22(50.0%)	15/31(48.4%)	20/37(54.1%)	11/38(28.9%)	0.14	0.35
Clinical pregnancy rate	8(57.1%)	12(63.2%)	15(68.2%)	10(43.5%)	0.37	0.35
Live birth rate	8(57.1%)	11(57.9%)	14(63.6%)	8(34.8%)	0.23	0.21

Note: continuous data are presented as mean ± SD and categoric data are presented as n (%). The P value is the value for subgroups comparison and P value with bold text means $P < 0.05$. P^a: uncontrolled for any indicators. P^b: controlled for maternal age, basal FSH level, AMH, LH and P on triggering day, oocytes retrieved, MII oocytes, 2PN embryos and good-quality embryos, Luteal phase support schemes.

for NOR, and 0.86–1.35 for POR to obtain the highest clinical pregnancy rate and live birth rate. This approach can help avoid excessive suppression or inadequate suppression. These findings provide a reference to control the LH suppression threshold during COS for IVF patients following the GnRH-A protocol to realize individualized patient management clinically.

Distinct clinical characteristics were observed among patients with different ovarian responses. Previous studies reported that

Table 4
Reproductive outcomes for patients with POR.

	Ratio of basal LH to LH on triggering day (bLH/hLH)				P value	
Outcomes	Q1(40) (<0.86)	Q2(40) (0.86–1.35)	Q3(40) (1.35–2.32)	Q4(39) (>2.32)	P^a	P^b
Oocytes retrieved	2.80 ± 2.45	2.75 ± 1.58	4.50 ± 2.73	4.10 ± 1.86	**<0.001**	**<0.001**
MII oocytes	2.57 ± 2.36	2.67 ± 1.53	4.28 ± 2.54	3.74 ± 1.86	**<0.001**	**<0.001**
MII oocyte rate	0.85 ± 0.33	0.95 ± 0.17	0.96 ± 0.10	0.89 ± 0.22	0.13	0.06
2PN embryos	1.78 ± 1.97	1.90 ± 1.32	2.93 ± 2.23	2.49 ± 1.39	**<0.05**	**<0.001**
2PN embryo rate	0.55 ± 0.43	0.71 ± 0.32	0.63 ± 0.31	0.62 ± 0.28	0.38	0.24
Good-quality embryos	1.10 ± 1.25	1.18 ± 1.19	1.55 ± 1.47	1.36 ± 1.48	0.45	**<0.001**
Good-quality embryo rate	0.39 ± 0.40	0.44 ± 0.38	0.36 ± 0.29	0.30 ± 0.31	0.49	0.42
Fresh embryo transfer rate	13(32.5%)	14(35.0%)	17(42.5%)	18(46.2%)	0.57	0.56
Embryo implantation rate	2/23(8.7%)	8/23(34.8%)	8/29(27.6%)	6/31(19.4%)	0.17	0.11
Clinical pregnancy rate	1(7.7%)	8(57.1%)	6(35.3%)	5(27.8%)	**<0.05**	0.11
Live birth rate	0(0.0%)	6(42.9%)	4(23.5%)	4(22.2%)	**<0.05**	0.94
Frozen embryo transfer rate	6(15.0%)	10(25.0%)	11(27.5%)	12(30.8%)	0.39	0.83
Embryo implantation rate	2/9(22.2%)	7/15(46.7%)	6/19(31.6%)	6/20(30.0%)	0.61	0.24
Clinical pregnancy rate	2(33.3%)	6(60.0%)	5(45.5%)	5(41.7%)	0.74	0.24
Live birth rate	2(33.3%)	5(50.0%)	4(36.4%)	4(33.3%)	0.85	0.87

Note: continuous data are presented as mean ± SD and categoric data are presented as n (%). The P value is the value for subgroups comparison and P value with bold text means $P < 0.05$. P^a: uncontrolled for any indicators. P^b: controlled for AMH, maternal age, basal LH level, LH, P and E_2 on triggering day, oocytes retrieved, MII oocytes, 2PN embryos and good-quality embryos.

patients with HOR tended to be younger with higher basal LH and T levels, higher AMH, and a higher AFC alongside a longer menstrual cycle. However, due to the heightened sensitivity of the ovary to Gn stimulation, these patients were at greater risk of OHSS and had a lower fresh embryo transfer rate than patients with NOR [30,36,37]. Patients with POR tended to be older with a lower AFC, lower AMH levels, and higher basal FSH levels. Because their ovaries respond poorly to Gn stimulation, these patients typically had fewer oocytes retrieved than NOR patients [30,38–41]. In this study, the clinical characteristics of HOR, NOR, and POR were consistent with previous findings, which further validated the reliability of our data for subsequent analyses. In the POR group, patients had a higher MII oocyte rate, 2PN embryo rate, and good-quality embryo rate, but the clinical pregnancy rate was lower than that in NOR. This may have been due to the advanced age of POR patients which can lead to decreased developmental potential of oocytes [42].

GnRH antagonists can be used to prevent the LH surge during COS without causing hypo-oestrogenic side effects, flare-ups, or long downregulation periods. These antagonists directly and rapidly inhibit gonadotrophin release within several hours by competitively binding to pituitary GnRH receptors. This property allows their use at any time during the follicular phase for patients with different ovarian responses [43,44]. The secretion and response of LH levels to GnRH antagonists vary widely among individuals, which means that the addition of GnRH antagonists might be tailored based on LH levels during COS. Studies have found that low LH levels (LH < 0.68 IU/L on triggering day or LH_{max} < 4 IU/L) during COS with the GnRH-A protocol decreased the ongoing pregnancy rate and live birth rate but increased the early miscarriage rate [31,45]. Notably, elevated LH levels during COS could promote follicular luteinization and negatively impact pregnancy outcomes [10,46], which is coincident with our study's findings. Whether in the HOR, NOR or POR group, the clinical pregnancy rate and live birth rate were lower in the Q1 and Q4 subgroups. In Q1, the bLH/hLH ratio was lower, which means that the LH level was higher on triggering day. In contrast, the LH level was lower on triggering day in Q4. In HOR, the clinical pregnancy rate was 68.4% ($P < 0.05$) in Q3, and the clinical pregnancy rate was 32.1% in Q1 and 33.3% in Q4 which were 36.3% and 35.1% lower respectively than those in Q3. The live birth rates were 17.9% in Q1 and 33.3% in Q4, which were 29.7% and 14.3% lower respectively than those in Q3. In the NOR group, the clinical pregnancy rate was 61.5% in Q2, 41.2% in Q1, and 38.1% in Q4, with the latter two rates being 20.3% and 23.4% lower respectively than those in Q2. The live birth rates were 30.6% in Q1 and 33.3% in Q4, which were 20.2% and 17.5% lower, respectively than those in Q2. In the POR group, the clinical pregnancy rate was 57.1% in Q2, which was significantly higher than those in Q1 (7.7%) and Q4 (27.8%) ($P < 0.05$). The live birth rates were 0.0% in Q1 and 22.2% in Q4 which were 42.9% and 20.7% lower than those in Q2 respectively. These results suggested that adequate follicular development and oocyte maturation require a certain threshold of endogenous LH and that severe suppression of LH levels may result in poor reproductive outcomes.

The sensitivity of ovaries to gonadotrophin stimulation differed among patients with HOR, NOR and POR, resulting in variations in the number of follicles that developed and in E_2 levels. E_2 levels regulate LH and FSH secretion through positive and negative feedback, leading to differences in LH level fluctuations during COS for HOR, NOR and POR patients following the GnRH-A protocol. In this study, the bLH/hLH ratio represented the suppression of LH levels during COS. The bLH/hLH ratio was highest in the HOR group and lowest in the POR group. Hence, the effect of the bLH/hLH ratio on reproductive outcomes should be analysed in NOR, HOR and POR. Previous studies have shown that serum LH levels can influence IVF outcomes [44,47–49], making LH levels during COS a potential indicator for timing and dose of antagonist administration. The decision to administer an antagonist depended on the NOR patient's LH level. Briefly, no antagonist was administered if the LH level was ≤4 IU/L; 0.125 mg antagonist was administered daily for 2 days until the next blood test, if the LH level was >4 IU/L but ≤6 IU/L. If the LH level was >6 IU/L or ≤10 IU/L, 0.25 mg antagonist was administered daily for 2 days. An antagonist (0.375 mg) was administered for 1 day if the LH level was >10 but ≤15 IU/L. An antagonist (0.5 mg) was administered for 1 day if the LH level >15 IU/L. The decision to continue antagonist administration depended on LH level >4 IU/L. Patients in the control group were administered a flexible GnRH antagonist protocol. They received 0.25 mg

antagonist daily until the trigger day if at least one follicle had reached a diameter of 14 mm [48,49]. The study found that the LH-based modified GnRH-A protocol was not inferior in clinical efficacy and resulted in higher cumulative and less financial expenditure for NOR patients. However, the multicentre randomized controlled trial investigated only the reproductive outcomes of NOR patients, and did not elucidate the timing and dose of GnRH antagonist addition in HOR and POR patients. Our study investigated the effect of suppression of LH levels on reproductive outcomes, provided LH suppression thresholds for patients with NOR, HOR or POR, and provided a more comprehensive and precise reference for clinicians to manage patients following the GnRH-A protocol individually according to LH levels in patients with differing ovarian responses.

In the NOR group, the embryo implantation rate, clinical pregnancy rate and live birth rate of ET were highest in Q2 ($P < 0.05$). The live birth rate of FET was also highest in Q2 ($P < 0.05$). The embryo implantation and clinical pregnancy rates of FET were highest in Q2, but the difference was not significant. In the HOR group, the embryo implantation, clinical pregnancy and live birth rates of ET were highest in Q3 ($P < 0.05$). The embryo implantation, clinical pregnancy and live birth rates of FET were highest in Q3, and the difference was not significant. Similarly, in the POR group, the clinical pregnancy and live birth rates of ET were highest in Q2 ($P < 0.05$). The embryo implantation, clinical pregnancy and live birth rates of FET were highest in Q2; however, there were no significant differences among the four groups. We speculate that the different effects of bLH/hLH on ET and FET pregnancy outcomes may be due to the impact on endometrial receptivity. Previous studies showed that FET could optimize endometrial receptivity [50–52]. Recent evidence has shown that IVF pregnancies conceived after frozen blastocyst transfer, compared to fresh pregnancies, have a lower uterine artery pulsatility index at 7–37 weeks gestation and a lower rate of small for gestational age (SGA) [53]. In the present study, there was only one POR cycle performed with fresh blastocyst transfer, and the patient did not become pregnant. The blastocyst implantation rate of FET was not significantly different among the three groups. Therefore, for patients undergoing ET, it is best to transfer embryos at the cleavage stage. If fresh blastocyst transfer is performed, foetal surveillance should be strengthened, and timely intervention should be taken if decreased uterine artery blood flow perfusion is observed to reduce the risk of SGA.

The present study has several limitations. First, it was retrospective, and a multicentre randomized controlled trial is still needed to validate the LH suppression thresholds for patients with NOR, HOR or POR. Second, the proportion of fresh embryo transfer cycles was relatively low in the HOR and POR groups, as clinicians tended to follow the freeze-all strategy to ensure the safety of HOR and POR patients. Third, the study was able to provide a range of LH suppression thresholds according to the quartile of the bLH/hLH ratio but not a definitive cut-off value. To address these issues, more clinical data on embryo transfer cycles will need to be collected in future research to further demonstrate the LH suppression threshold in HOR and POR. On this basis, the data could lead to a more precise cut-off value for the LH suppression threshold for HOR, POR and NOR patients.

5. Conclusion

In summary, our study demonstrated that suppression of LH levels in the GnRH-A protocol can impact reproductive outcomes for patients with different ovarian responses. By using AMH levels, clinicians can preliminarily classify patients' ovarian responses (HOR, NOR and POR) before starting COS. The bLH/hLH ratio was found to vary significantly among patients with NOR, HOR and POR and was identified as a more sensitive indicator for monitoring LH levels during COS in patients following the GnRH-A protocol. The use of LH suppression thresholds of 2.40–3.69 for HOR, 1.29–2.05 for NOR, and 0.86–1.35 for POR could benefit patients, and these thresholds could serve as helpful guidelines for clinicians to adjust LH levels during COS and manage patients following the GnRH-A protocol individually.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Women's Hospital of Zhejiang University (reference: IRB-20210015-R). Written informed consent for participation was required for this study in accordance with the national legislation and the institutional requirements.

Consent for publication

Not applicable.

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Data availability statement

Data will be made available on request. Further inquiries can be directed to the corresponding author.

CRediT authorship contribution statement

Qingfang Li: Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Xiaoqian Zhou:** Writing – review & editing, Formal analysis, Data curation. **Bingru Ye:** Writing – review & editing. **Minyue Tang:** Writing – review & editing,

Conceptualization. **Yimin Zhu:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Yimin Zhu reports was provided by None. Yimin Zhu reports a relationship with None that includes:. None has patent pending to None. The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23933.

Abbreviation

LH	luteinizing hormone
NOR	normal ovarian response
HOR	high ovarian response
POR	poor ovarian response
IVF	in vitro fertilization
GnRH	gonadotropin-releasing hormone
HCG	human chorionic gonadotropin
MII	mature oocytes
2PN	two primary pronucleus embryo
COS	controlled ovarian stimulation
GnRH-A:	gonadotropin-releasing hormone antagonist
GnRH-a:	gonadotropin-releasing hormone agonist
FSH	follicle stimulating hormone
OHSS	ovarian hyperstimulation syndrome
AFC	antral follicle count
BMI	body mass index
E_2	Estradiol
P	progestin
T	Testosterone
AMH	Anti-Mullerian hormone
bLH	Basal LH level
hLH	LH level on triggering day
SGA	small for gestational age

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