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Effect of LH level on HCG trigger day on clinical outcomes in patients with diminished ovarian reserve undergoing GnRH-antagonist protocol

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Abstract

Research question Does luteinizing hormone (LH) levels on human chorionic gonadotropin (HCG) trigger day (LH_{HCG}) affect the clinical outcomes of patients with diminished ovarian reserve (DOR) undergoing gonadotropin-releasing hormone antagonist (GnRH-ant) protocol?

Methods Retrospective analysis fresh embryo transfer cycles of DOR patients who underwent GnRH-ant protocol from August 2019 to June 2023. The participants were divided into different groups according to LH_{HCG} level and age. The clinical data and outcomes were compared between groups.

Results In patients with DOR, the HCG positive rate (59.3% versus 39.8%, $P=0.005$), embryo implantation rate (34.5% versus 19.7%, $P=0.002$), clinical pregnancy rate (49.2% versus 28.4%, $P=0.003$), live birth rate (41.5% versus 22.7%, $P=0.005$) in LH_{HCG} < 2.58 IU/L group were significantly higher than LH_{HCG} ≥ 2.58 IU/L group. There was no significant correlation between LH_{HCG} level and clinical pregnancy in POSEIDON group 3. In POSEIDON group 4, the HCG positive rate (52.8% versus 27.0%, $P=0.015$), embryo implantation rate (29.2% versus 13.3%, $P=0.023$), clinical pregnancy rate (45.3% versus 18.9%, $P=0.010$) in LH_{HCG} < 3.14 IU/L group were significantly higher than LH_{HCG} ≥ 3.14 IU/L group. Logistic regression analysis indicated that LH_{HCG} level was an independent influencing factor for clinical pregnancy in POSEIDON group 4 patients (OR = 3.831, 95% CI: 1.379–10.643, $P<0.05$).

Conclusions LH_{HCG} level is an independent factor affecting pregnancy outcome of fresh embryo transfer in DOR patients undergoing GnRH-ant protocol, especially for advanced-aged women. LH_{HCG} had a high predictive value for POSEIDON group 4 patients, and LH_{HCG} ≥ 3.14 IU/L predicts poor pregnancy outcomes.

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Keywords Luteinizing hormone on HCG day, Diminished ovarian reserve, GnRH-antagonist protocol, POSEIDON criteria

Introduction

Diminished ovarian reserve (DOR) is one of the common causes of female infertility which refers to reducing the quantity and quality of oocytes. According to the research conducted by the US-based National Society for Assisted Reproductive Technology (ART) in 2014, 32% of IVF patients can be diagnosed as DOR [1]. The demand for assisted reproduction among DOR women with reduced fertility has increased significantly. At present, there is no unified opinion on the diagnosis of DOR. The POSEIDON criteria proposed in 2016 divides DOR people into groups according to age and ovarian reserve parameters included antral follicle count (AFC) and anti-Müllerian hormone (AMH), which improves the homogeneity and comparability of clinical studies and is widely used in clinical studies [2].

The gonadotropin-releasing hormone antagonist (GnRH-ant) protocol is the current first-line clinical regimen for promoting ovulation, and it is also applicable to DOR patients [3]. Compared with the GnRH agonist protocol, it can significantly shorten the treatment time, reduce the dosage of gonadotropin, avoid excessive pituitary suppression, and reduce the incidence of severe ovarian hyperstimulation syndrome (OHSS) [4]. Luteinizing hormone (LH) plays an important role in follicular development, ovulation and steroid production, and affects luteal function and endometrial development. Studies have shown that maintaining a reasonable LH level during ovulation induction therapy is beneficial to improve the pregnancy outcome of in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycle [5, 6].

LH levels on human chorionic gonadotropin (HCG) trigger day (LH_{HCG}) play an important predictive role in the treatment of ovulation induction with IVF. The research by Jin et al. has shown that high level of LH_{HCG} in GnRH-ant protocol predicts significantly higher oocyte retrieval rate and mature oocyte retrieval rate compared with low—medium levels [7]. The study by Shi et al. showed that LH_{HCG} has important predictive value for pregnancy outcome of frozen embryo transfer in an ovulation-induced cycle for endometrial preparation [8]. In addition, research has shown that LH_{HCG} has specific predictive value for the pregnancy outcome of patients with polycystic ovary syndrome (PCOS) and normal ovarian responders (NOR), and low LH_{HCG} level indicates poor pregnancy outcome [9]. The study by Luo et al. has shown that LH_{HCG} in the GnRH agonist protocol was positively correlated with the clinical pregnancy and live birth rates, while in the GnRH-ant protocol, LH_{HCG} was only positively correlated with clinical pregnancy rate of young women (<35 years) [10].

Nonetheless, up to now no related researches discuss whether the LH_{HCG} in patients with DOR has a predictive value on pregnancy outcome. Therefore, the objective of our study was to explore the effect of LH_{HCG} on the clinical pregnancy outcomes of patients with DOR, which can provide evidence for clinical diagnosis and treatment.

Materials and methods

Study design and patients

This is a retrospective study, DOR patients who underwent GnRH-ant protocol in the Reproductive Medical Center of Renmin Hospital of Wuhan University were enrolled from August 2019 to June 2023. The study conformed to the 'Declaration of Helsinki for Medical Research involving Human Subjects'. Also, This study was approved by the Ethical Committee of Renmin Hospital of Wuhan University (Protocol #: 2023 K-K192).

The DOR patients were included in this study according to POSEIDON criteria, including POSEIDON group 3 (G3, < 35 years, AMH<1.2 ng/ml and/or AFC<5) and POSEIDON group 4 (G4, ≥ 35 years, AMH<1.2 ng/ml and/or AFC<5) [2, 11]. Inclusion criteria were as follows: (i) Age<45 years; (ii)AFC<5 or AMH<1.2 ng/ml; (iii) body mass index (BMI)<28 kg/m^2; (iv) fresh embryo transfer cycle; (v) estrogen (E_2)<3200 pg/ml, progesterone (P)<1.2 ng/ml, and endometrial thickness≥7 mm on the HCG trigger day. Patients diagnosed with uterine abnormalities, hyperprolactinemia, endometriotic cyst, hydrosalpinx and congenital adrenal hyperplasia, thyroid disease or use of oral contraceptive, chromosome abnormality were excluded. Patients with previous IVF cycles≤3 were included in this study. A total of 206 oocyte retrieval cycles were included according to the above inclusion and exclusion criteria as Fig. 1.

Clinical setting

In this study, all patients received the GnRH-ant protocol. Recombinant follicle-stimulating hormone (rFSH, Gonal-f, Merck Serono, Darmstadt, Germany) was used on the second or third day of menstrual cycle, and the initial dose was performed according to the patient's age, BMI, AFC. B-ultrasound examination and sex hormone were used to monitor follicular growth to adjust the dose of gonadotropin (Gn). When the follicle mean diameter reached 14 mm or E_2 serum levels>300 pg/ml; GnRH-ant (Cetrotide, Merck, Kenilworth, NJ, USA) was injected at 0.25 mg/day until HCG trigger day. When more than two follicles' diameter reached 18 mm, final

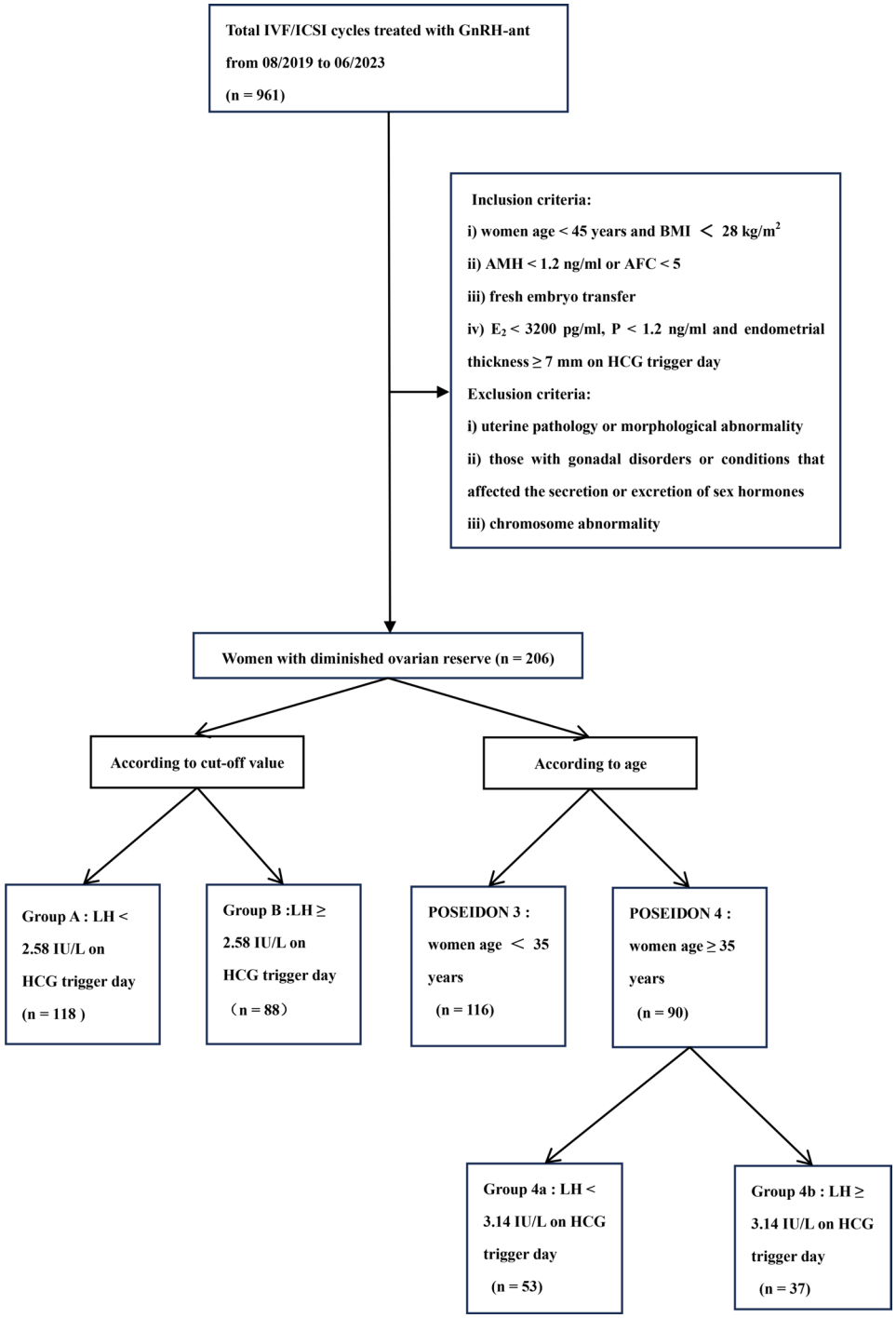

Fig. 1 The flow chart of study

oocyte maturation was triggered by 0.2 mg GnRH agonist (Decapeptyl 0.2 mg, Ferring International Center SA, Kiel, Germany) and 250 ug HCG (Lizhu Pharmaceutical Factory, China) [12].

Blood sample and hormone assays

According to the routine clinical procedure of our center, the serum samples of all patients were taken between 8: 00 am and 10: 00 am. The serum hormone levels were detected for the first time on the second or third day of the menstrual cycle, and then once every 1 to 2 days according to the follicular development, until the HCG

day. The serum LH level on the HCG day was defined as LH_{HCG}.

Embryo transfer and follow-up

Oocytes retrieval were performed 36–38 h after HCG administration by the guide of transvaginal ultrasonography. Oocytes were inseminated either by IVF or ICSI according to the quality of sperm. In this study, all embryo transfer were conducted on day 3 after oocyte retrieval and a maximum of two embryos were transferred. A high-quality embryo on day 3 was defined as an embryo with seven or eight blastomeres, no multinucleation and <20% fragmentation. The luteal phase support was started on the day of oocyte retrieval. Intramuscular progesterone (20 mg once daily), vaginal progesterone sustained-release gel (Crinone 8%, 90 mg once daily) and oral progesterone (Dydrogesterone, 20 mg twice daily) was administered until 10 weeks of gestation. A serum β-HCG test was performed 12 days after embryo transfer. Serum β-HCG>10 IU/L was considered a chemical pregnancy. Clinical pregnancy was defined as the presence of a gestational sac with a fetal heart under ultrasonography 30 days after embryo transfer [12].

Outcome measures and definition

The baseline characteristics, number of oocytes retrieved, 2PN fertilization rate, cleavage rate, high-quality embryo rate, HCG positive rate, embryo implantation rate, clinical pregnancy rate, early miscarriage rate, and live birth rate were compared in each group. In addition, the number of follicles≥14 mm on HCG trigger day was calculated as an index of ovarian response [13, 14].

Statistical analysis

SPSS 25.0 (IBM Corp., USA) was used for data analysis. Receiver Operating Characteristic (ROC) was performed to analyze the relationship between LH_{HCG} and clinical pregnancy rate in patients with DOR. The cut-off value was determined according to the maximum value of Youden index, and the Youden index is equal to sensitivity+specificity −1. Measurement data conforming to a normal distribution were expressed as mean±SD, and the independent sample t-test was used to compare variables between groups. Enumeration data were expressed as frequency (%). Categorical variables were compared using the chi-square test or Fisher's precision probability test. The binary logical regression model is used to test whether LH_{HCG} level is an independent influencing factor of clinical pregnancy. Bonferroni correction was used for multiple comparisons of data. The p-value<0.05 was considered statistically significant.

Results

A total of 206 IVF/ICSI cycles were included in this study. ROC curve was used to analyze the relationship between LH_{HCG} level and clinical pregnancy rate of DOR patients, and the result was shown in curve A of Fig. 2. The area under curve (AUC) of the LH_{HCG} level for predicting the clinical pregnancy outcome was 0.610 (95%CI: 0.531~0.689, $P < 0.05$). According to the Youden index, the cut-off value of ROC curve A is 2.58 IU/L, and the LH_{HCG} has the highest sensitivity and specificity (51.2% and 69.9%, respectively) in predicting clinical pregnancy.

According to the cut-off value of the ROC curve A, patients with DOR were divided into group A (LH_{HCG} < 2.58 IU/L, $n=118$) and group B (LH_{HCG} ≥ 2.58 IU/L, $n=88$). The comparison of baseline characteristics and controlled ovarian stimulation characteristics of patients between the two groups is shown in Table 1. The basal FSH and basal LH in group A were significantly lower than those in group B ($P<0.05$), while the number of follicles≥14 mm on HCG day, AFC, and AMH in group A were significantly higher than those in group B ($P<0.05$). There was no significant difference in other characteristics between groups A and B.

The comparison of laboratory parameters and clinical outcomes between groups A and B is shown in Table 2. The number of oocytes retrieved and MII oocytes in group A were significantly higher than those in group B ($P<0.05$). The comparison of clinical outcomes indicated that the HCG positive rate (59.3% versus 39.8%, $P=0.005$), embryo implantation rate (34.5% versus 19.7%, $P=0.002$), clinical pregnancy rate (49.2% versus 28.4%, $P=0.003$), and the live birth rate (41.5% versus 22.7%, $P=0.005$) in group A were significantly higher than those in group B. There was no statistically significant difference in the number of transferred embryos and early miscarriage rate between group A and group B. Logistic regression analysis showed that LH_{HCG} was an independent influencing factor of clinical pregnancy in patients with DOR (OR=2.007, 95%CI: 1.052 ~ 3.830, $P < 0.05$), as shown in Table 3.

According to the POSEIDON criteria, patients were further divided into POSEIDON group 3 (<35, $n=116$) and POSEIDON group 4 (≥35, $n=90$). The ROC curves of LH_{HCG} and clinical pregnancy rate were shown in Fig. 2.

In POSEIDON group 3 patients, curve B indicated that the AUC of the LH_{HCG} level for predicting the clinical pregnancy outcome was 0.556 (95%CI: 0.048~0.663, $P>0.05$), which was not statistically significant. For POSEIDON group 4, curve C indicated that the AUC of the LH_{HCG} level for predicting the clinical pregnancy outcome was 0.672 (95%CI: 0.558~0.786, $P < 0.05$), and the cut-off value is 3.14 IU/L. At an cut-off value of 3.14 IU/L, the sensitivity and specificity of LH_{HCG} were 50.8%

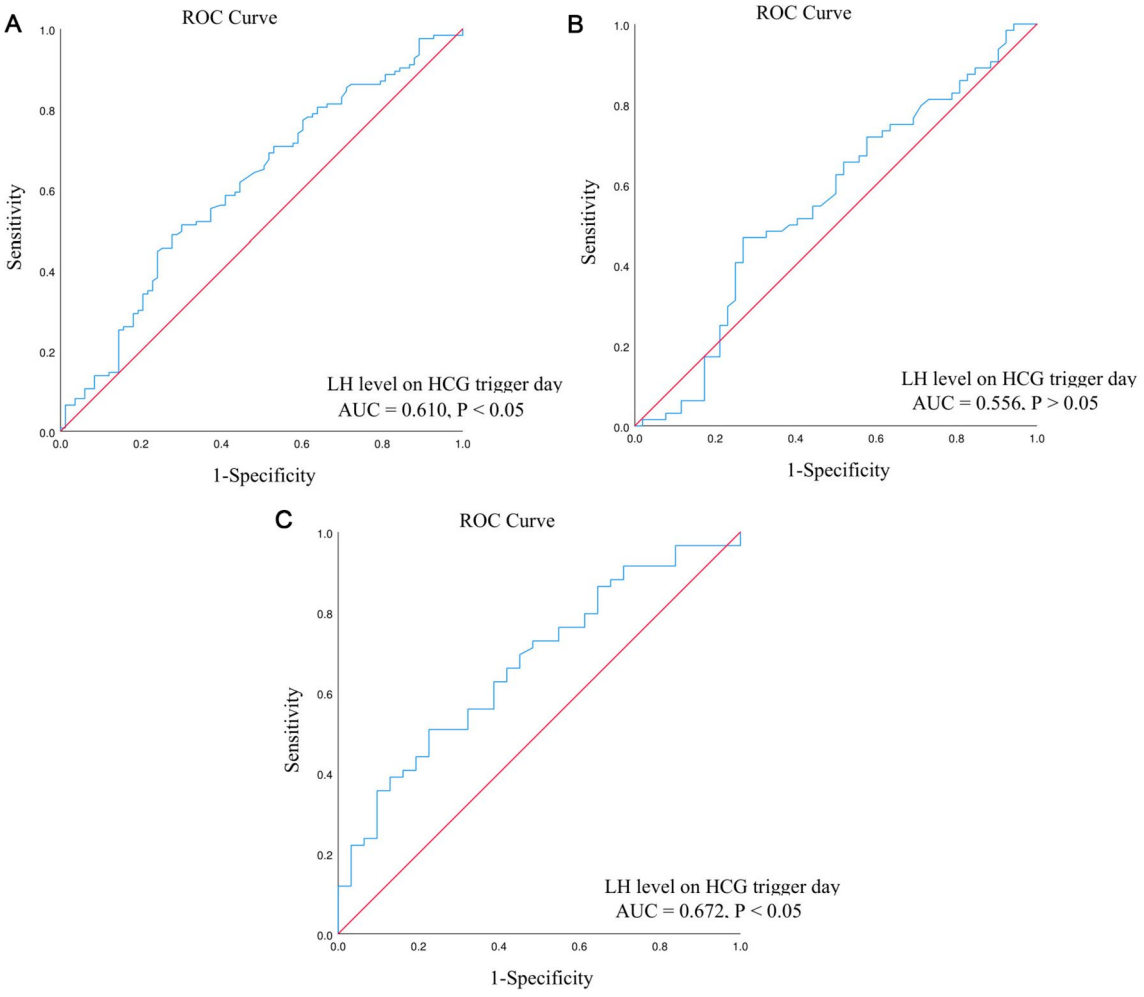

Fig. 2 ROC curve (**A**): analyze the relationship between LH_{HCG} level and clinical pregnancy rate of DOR patients. (**B**): analyze the relationship between LH_{HCG} level and clinical pregnancy rate of POSEIDON group 3. (**C**): analyze the relationship between LH_{HCG} level and clinical pregnancy rate of POSEIDON group 4

and 77.4%, respectively. According to the cut-off value, POSEIDON group 4 patients were divided into group G4a (LH_{HCG} < 3.14 IU/L, n=53) and group G4b (LH_{HCG} ≥ 3.14 IU/L, n=37).

A comparison of G4a and G4b groups was carried out to examine baseline characteristics and controlled ovarian stimulation characteristics, which were shown in Table 4. The number of follicles≥14 mm on HCG day in group G4a was more than that in group G4b (P<0.05), and no significant difference was found in other characteristics. The comparison of laboratory parameters and clinical outcomes between group G4a and G4b is shown in Table 5. Compared to the LH_{HCG} ≥ 3.14 IU/L group, the HCG positive rate (52.8% versus 27.0%, P=0.015), embryo implantation rate (29.2% versus 13.3%, P=0.023), and clinical pregnancy rate (45.3% versus 18.9%, P=0.010) were significantly higher in LH_{HCG} < 3.14 IU/L group. There was no significant difference in other characteristics. Logistic regression analysis showed that LH_{HCG} was an independent influencing factor of clinical pregnancy in patients with POSEIDON group 4 (OR=3.831, 95%CI: 1.379～10.643, P<0.05), as shown in Table 6.

Discussion

The purpose of this study is to investigate the effect of LH_{HCG} on clinical pregnancy outcome in patients with DOR undergoing GnRH-ant protocol, and our results showed that LH_{HCG} level was an independent factor affecting the clinical pregnancy outcome of patients with DOR. The HCG positive rate, embryo implantation rate, clinical pregnancy rate, and live birth rate in LH_{HCG} < 2.58 IU/L group were significantly higher than those in LH_{HCG} ≥ 2.58 IU/L group. However, in the study of Zhou et al., the live birth rate of the low LH_{HCG} level group was lower than that of the high-level group among the NOR and PCOS population who used GnRH-ant protocol for fresh embryo transfer, while there was no significant

Table 1 The comparison of baseline characteristics and ovulation induction between Group A and Group B

Variables	Group A LH_{HCG} < 2.58 IU/L	Group B LH_{HCG} ≥ 2.58 IU/L	*P*-value
Age (years)	33.79 ± 4.81	34.44 ± 5.17	0.350
Infertility duration (years)	3.34 ± 2.80	3.30 ± 2.76	0.912
BMI (kg/m^2)	22.02 ± 2.62	21.93 ± 2.59	0.797
Basal FSH (IU/L)	9.17 ± 3.50	10.78 ± 3.56	0.001
Basal LH (IU/L)	3.12 ± 1.43	3.70 ± 1.65	0.007
Basal E_2 (pg/ml)	54.16 ± 102.01	45.55 ± 21.77	0.437
AFC	7.74 ± 3.19	6.61 ± 3.07	0.012
AMH (ng/ml)	0.75 ± 0.26	0.63 ± 0.29	0.002
Fertilization type			0.418
IVF	84/118 (71.2)	58/88 (65.9)	
ICSI	34/118 (28.8)	30/88 (34.1)	
Gn total dosage (IU)	2474.41 ± 632.66	2483.13 ± 836.48	0.932
Gn duration (days)	9.42 ± 1.76	9.52 ± 2.33	0.739
Trigger day			
Number of follicles ≥ 14 mm	5.11 ± 2.36	3.69 ± 1.82	<0.001
Endometrial thickness (mm)	11.40 ± 2.14	11.17 ± 2.02	0.449
E_2 (pg/ml)	1443.11 ± 758.17	1310.49 ± 627.39	0.172
Progesterone (ng/ml)	0.65 ± 0.23	0.67 ± 0.22	0.499

Date are shown as mean ± SD or n (%)

BMI: Body mass index; FSH, follicle stimulating hormone; LH, luteinizing hormone; E_2: Estrogen; AFC: Antral follicle count; AMH: Anti-Müllerian hormone; IVF, vitro fertilization; ICSI, intracytoplasmic sperm injection; Gn: Gonadotropin

Table 3 Logistic regression analysis of clinical pregnancy rate in patients with DOR

Variables	Adjusted OR	95%CI	*P*-value
Basal FSH (IU/L)	0.948	0.857~1.049	0.302
Basal LH (IU/L)	0.829	0.660~1.043	0.109
AFC	0.880	0.780~0.993	0.038
AMH (ng/ml)	1.629	0.482~5.503	0.432
Number of follicles ≥ 14 mm	0.911	0.738~1.125	0.388
Number of oocytes retrieved	1.159	0.915~1.466	0.221
Number of MII oocyte	1.065	0.827~1.372	0.625
LH groups	2.007	1.052~3.830	0.035

FSH, follicle stimulating hormone; LH, luteinizing hormone; AFC: Antral follicle count; AMH: Anti-Müllerian hormone

Table 2 The comparison of laboratory parameters and clinical outcomes between Group A and Group B

Variables	Group A LH_{HCG} < 2.58 IU/L	Group B LH_{HCG} ≥ 2.58 IU/L	*P*-value
Number of oocytes retrieved	5.48 ± 2.88	4.01 ± 2.38	<0.001
Number of MII oocyte	4.06 ± 2.41	3.11 ± 1.83	0.002
2PN fertility rate	386/647 (59.7)	220/353 (62.3)	0.875
2PN Cleavage rate	380/386 (98.4)	218/220 (99.1)	0.094
Number of high-quality embryos	1.78 ± 1.54	1.53 ± 1.39	0.231
High-quality embryo rate	210/386 (54.4)	133/220 (60.5)	0.504
Number of embryo transfer	1.72 ± 0.45	1.69 ± 0.47	0.635
HCG positive rate	70/118 (59.3)	35/88 (39.8)	0.005
Implantation rate	70/203 (34.5)	29/147 (19.7)	0.002
Clinical pregnancy rate	58/118 (49.2)	25/88 (28.4)	0.003
Early miscarriage rate	5/58 (8.6)	4/25 (16.0)	0.544
Live birth rate	49/118 (41.5)	20/88 (22.7)	0.005

Date are shown as mean ± SD or n (%)

MII oocyte: oocytes in the metaphase of the second meiosis

2PN: two pronuclear fertilized eggs

HCG positive rate = number of HCG positive cycles/number of transfer cycles × 100%,

Implantation rate = number of pregnancy sacs/number of transferred embryos × 100%,

Clinical pregnancy rate = number of clinical pregnancy cycles/number of transfer cycles × 100%,

Early miscarriage rate = number of early miscarriage cycles/clinical pregnancy cycles × 100%

Live birth rate = number of live birth cycles/number of transfer cycles × 100%

difference in POR patients, which was different from our results [9]. This may be due to research population's different inclusion criteria and grouping methods. The Bologna criteria was adopted in the study of Zhou et al., while the POSEIDON criteria was adopted in our study.

With the rapid development of ART, the GnRH-ant protocol has been commonly used around the world. In 2020, the European Society for Human Reproduction and Embryology (ESHRE) issued controlled ovarian hyperstimulation (COH) guidelines, which indicated that GnRH-ant protocol can be used as first-line treatment for normal ovarian response, diminished ovarian reserve, and patients with high ovarian response [15]. GnRH-ant protocol competitively blocks pituitary GnRH receptors and inhibits premature LH surge, which can prevent premature ovulation [16, 17]. Studies have indicated that there was no significant difference in clinical pregnancy rate and live birth rate between the GnRH-ant protocol and GnRH agonist protocol, and the number of high-quality embryos was similar. But compared to the GnRH agonist protocol, the GnRH-ant protocol had a lower implantation rate, clinical pregnancy rate and cumulative pregnancy rate [18–20]. Therefore, improving the implantation rate and pregnancy rate of patients is one of the key points in the application of the GnRH-ant protocol.

LH level is one of the factors that affecting COH and pregnancy outcome in GnRH-ant protocol [21]. LH controls gonadal function and plays a central role in regulating the complicated and delicate endocrine mechanisms of ovarian biology [22]. It controls the length and sequence of women's menstrual cycle, including

Table 4 The comparison of baseline characteristics and ovulation induction between Group 4a and Group 4b

Variables	Group 4a LH_{HCG} < 3.14 IU/L	Group 4b LH_{HCG} ≥ 3.14 IU/L	*P*-value
Age (years)	38.45 ± 2.70	39.08 ± 3.12	0.311
Infertility duration (years)	3.81 ± 3.36	3.97 ± 3.58	0.827
BMI (kg/m^2)	21.78 ± 2.39	22.37 ± 2.70	0.279
Basal FSH (IU/L)	9.65 ± 3.82	11.07 ± 3.85	0.084
Basal LH (IU/L)	3.16 ± 1.55	3.73 ± 1.57	0.089
Basal E_2 (pg/ml)	68.08 ± 150.08	46.19 ± 24.20	0.383
AFC	6.74 ± 3.20	5.81 ± 2.76	0.157
AMH (ng/ml)	0.66 ± 0.28	0.58 ± 0.32	0.252
Fertilization type			0.309
IVF	37/53 (69.8)	22/37 (59.5)	
ICSI	16/53 (30.2)	15/37 (40.5)	
Gn total dosage (IU)	2525.57 ± 702.66	2489.80 ± 998.31	0.842
Gn duration (days)	9.36 ± 2.23	9.30 ± 2.84	0.909
Trigger day			
Number of follicles ≥ 14 mm	4.25 ± 2.16	3.16 ± 1.63	0.008 *
Endometrial thickness (mm)	11.50 ± 2.34	10.60 ± 2.06	0.063
E_2 (pg/ml)	1288.29 ± 741.92	1103.25 ± 586.81	0.191
Progesterone (ng/ml)	0.60 ± 0.23	0.65 ± 0.21	0.263

Date are shown as mean ± SD or n (%),

* After Bonferroni correction, $P < 0.025$

BMI: Body mass index; FSH, follicle stimulating hormone; LH, luteinizing hormone; E_2: Estrogen; AFC: Antral follicle count; AMH: Anti-Müllerian hormone; IVF, vitro fertilization; ICSI, intracytoplasmic sperm injection; Gn: Gonadotropin

Table 5 The comparison of laboratory parameters and clinical outcomes between Group 4a and Group 4b

Variables	Group 4a LH_{HCG} < 3.14 IU/L	Group 4b LH_{HCG} ≥ 3.14 IU/L	*P*-value
Number of oocytes retrieved	4.43 ± 2.29	3.57 ± 2.50	0.093
Number of MII oocyte	3.36 ± 1.97	2.72 ± 1.89	0.132
2PN fertility rate	145/235 (61.7)	83/132 (62.9)	0.748
2PN Cleavage rate	144/145 (99.3)	82/83 (98.8)	0.867
Number of high-quality embryos	1.66 ± 1.34	1.56 ± 1.30	0.715
High-quality embryo rate	88/145 (60.7)	56/83 (67.5)	0.253
Number of embryo transfer	1.68 ± 0.47	1.67 ± 0.48	0.902
HCG positive rate	28/53 (52.8)	10/37 (27.0)	0.015 *
Implantation rate	26/89 (29.2)	8/60 (13.3)	0.023 *
Clinical pregnancy rate	24/53 (45.3)	7/37 (18.9)	0.010 *
Early miscarriage rate	3/24 (12.5)	2/7 (28.6)	0.562
Live birth rate	19/53 (35.8)	5/37 (13.5)	0.034

Date are shown as mean ± SD or n (%),

*After Bonferroni correction, $P < 0.025$

MII oocyte: oocytes in the metaphase of the second meiosis

2PN: two pronuclear fertilized eggs

HCG positive rate = number of HCG positive cycles/number of transfer cycles × 100%,

Implantation rate = number of pregnancy sacs/number of transferred embryos × 100%,

Clinical pregnancy rate = number of clinical pregnancy cycles/number of transfer cycles × 100%,

Early miscarriage rate = number of early miscarriage cycles/clinical pregnancy cycles × 100%

Live birth rate = number of live birth cycles/number of transfer cycles × 100%

Table 6 Logistic regression analysis of clinical pregnancy rate in POSEIDON Group 4 patients

Variables	Adjusted OR	95%CI	*P*-value
Number of follicles ≥ 14 mm	0.933	0.740~1.178	0.561
LH groups	3.831	1.379~10.643	0.010

LH, luteinizing hormone

ovulation, the preparation of fertilized embryos for implantation into the uterus, and the production of estrogen and progesterone [23]. While clinical studies have shown that abnormal serum LH levels often occur in patients with DOR [24].

LH is essential for estrogen synthesis and maintaining the development of dominant follicles, and excessive stimulation of the ovaries by LH will adversely affect the normal development of pre-ovulatory follicles. Depending on the stage of development, exposure to inappropriately high concentrations of LH will interfere with communication between cumulus cells and granulosa cells, which affects the development of oocytes and may lead to follicular atresia or premature luteinization [25]. At present, the relationship between LH_{HCG} and ovarian reserve and ovarian response in DOR patients is not completely clear. In our study, the LH_{HCG} < 2.58 IU/L group had higher ovarian reserve and ovarian response than the LH_{HCG} ≥ 2.58 IU/L group. The AFC and AMH, and the number of oocytes retrieved, number of mature oocytes in LH_{HCG} < 2.58 IU/L group were more than those in LH_{HCG} ≥ 2.58 IU/L group. It suggests that LH_{HCG} may be an important indication of ovarian response. Xu et al. has confirmed that LH_{HCG} ≥ 2 IU/L in GnRH-ant protocol is not conductive to follicles maturation and ovulation [26]. The study by Zhang et al. showed that in the follicular-phase long protocol, the LH_{HCG} was negatively correlated with the number of oocytes retrieved, and the highest number of oocytes was retrieved when LH_{HCG} ≤ 0.5 IU/L [27]. The optimal range of LH_{HCG} in this study is different from that in previous studies, which may be due to the fact that this study only focused on DOR populations. This also suggests that conducting separate studies on different populations is beneficial to providing individualized treatment for patients during clinical ovulation induction.

The effect of LH on pregnancy outcomes and its mechanism are still not fully understood. Lucas et al. found

that LH can inhibit the proliferation, migration, and differentiation of endometrial stem cells through Akt and ERK1/2 signaling pathways, and inhibit tissue regeneration-related functions through its cognate receptor LHR, thus reducing endometrial receptivity [28]. Besides, a rise in LH is often accompanied by a rise in progesterone levels, and the study has shown that $LH_{HCG} \geq 8.46$ IU/L may indirectly affect endometrial receptivity through the increase of progesterone, which may lead to the decrease of pregnancy rate in frozen embryo transfer cycle [8]. The increase of progesterone concentration not only damages endometrial receptivity but also affects embryo quality [29]. Since there was no significant difference in the high-quality embryo rate and the number of embryos transferred between the $LH_{HCG} < 2.58$ IU/L group and the $LH_{HCG} \geq 2.58$ IU/L group in this study, it is speculated that the high level of LH_{HCG} may damage the endometrial receptivity and thus affect pregnancy outcomes. However, in our study, there was no significant difference in progesterone level on HCG trigger day between $LH_{HCG} < 2.58$ IU/L group and $LH_{HCG} \geq 2.58$ IU/L group, which suggests that progesterone-mediated endometrial receptivity may not be the direct cause of the effect of LH level on pregnancy outcomes, and the specific mechanism needs further study.

The POSEIDON criteria further stratified the patients with poor or suboptimal ovarian response based on the Bologna standard. The female age in the ART cycle is related to the embryo aneuploidy rate and also be a sign of oocyte quality in the POSEIDON criteria [3]. Therefore, in this study the patients were divided into two groups according to age according the POSEIDON criteria, and we found that LH_{HCG} level had a more significant predictive value for clinical pregnancy in advanced-aged women. Compared to the $LH_{HCG} \geq 3.14$ IU/L group, patients in the $LH_{HCG} < 3.14$ IU/L group had higher HCG positive rate, embryo implantation rate, and clinical pregnancy rate. This is consistent with the study by Gao et al., which showed that among advanced-aged patients (≥37 years), the cumulative live birth rate in the group with abnormally elevated LH_{HCG} was lower than normal LH_{HCG} group [30]. Erhan et al. found that the high level of LH in the late follicular phase leads to early luteinization of oocytes and premature maturity of the endometrium, which leads to an abnormal implantation environment, especially in the advanced-aged women [31]. This may be one of the reasons why LH_{HCG} is more significant in advanced-aged women and the pregnancy outcome in the group with high LH levels is poor in our study.

This was the first study to explore the predictive value of LH_{HCG} in pregnancy outcomes in DOR patients in IVF/ICSI, and we found that LH_{HCG} was an independent factor affecting clinical pregnancy rate in advanced-aged patients with DOR. Moreover, our results showed that high LH_{HCG} level in the antagonist regimen was not conducive to the pregnancy outcome of the fresh embryo transfer cycle in patients with DOR, which suggests that we need to focus on LH_{HCG} to comprehensively consider the feasibility of fresh embryo transfer.

Limitation

The main limitation of our study is that this is a retrospective study with inevitable selective bias. In addition, the sample size is limit, large-sample prospective trials and multicenter randomized controlled trials are still needed for further verification and clarify the specific mechanisms in the future.

Conclusion

In conclusion, the results demonstrate the important predictive value of LH_{HCG} level in GnRH-ant protocol for pregnancy outcomes in DOR patients. High LH_{HCG} level leads to poor pregnancy outcomes of fresh embryo transfer, especially for advanced-aged women. Therefore, for advanced-aged patients with DOR under the GnRH-ant protocol, we need to pay attention to the LH_{HCG} level.

Abbreviations

LH	Luteinizing hormone
HCG	Human chorionic gonadotropin
LH_{HCG}	Luteinizing hormone levels on human chorionic gonadotropin
DOR	Diminished ovarian reserve
GnRH-ant	Gonadotropin-releasing hormone antagonist
ART	Assisted Reproductive Technology
AFC	Antral follicle count
AMH	Anti-Müllerian hormone
OHSS	Ovarian hyperstimulation syndrome
IVF	In Vitro fertilization
ICSI	Intracytoplasmic sperm injection
PCOS	Polycystic ovary syndrome
NOR	Normal ovarian responders
BMI	Body mass index
E_2	Estrogen
P	Progesterone
Gn	Gonadotropin
ROC	Receiver operating characteristic
AUC	Area under curve
ESHRE	European Society for Human Reproduction and Embryology
COH	Controlled ovarian hyperstimulation

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Author contributions

Q.J.Z.: acquisition, analysis and interpretation of date; Y.G.: acquisition of data ; K.X.Z.: acquisition, analysis and interpretation of date, writing-original draft; S.J.H. and Y.C.M.: acquisition and analysis of date; T.L.Y, L.M. and J.Y.: the conception and design of the study; S.W., Z.M.Z. and W.L.: the design of the study and revising content; S.J.L.: the conception and design of the study, revising it critically for important intellectual content. All authors reviewed the manuscript.

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Data availability
The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Declarations

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Consent to participate
not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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