Original Article

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Women undergoing *in vitro* fertilization/intracytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET) might benefit from maintaining serum luteinizing hormone levels: A retrospective analysis

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SUMMARY We aimed to evaluate the effect of serum luteinizing hormone (LH) levels on human chorionic gonadotropin (HCG) injection day (LH_{HCG}) on outcomes of *in vitro* fertilization/intracytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET) patients. It is a retrospective cohort study involving 620 women who had an IVF cycle in Taizhou Hospital Affiliated to Wenzhou Medical University between 2018-2020. The participants were divided into different groups according to LH_{HCG} level and age. The clinical data and outcomes were compared between groups. The numbers of follicles (≥ 14 mm) on HCG day, retrieved oocytes, mature oocytes, and two pronuclei (2PN) embryos in women with LH_{HCG} < 2 IU/L were more than those with LH_{HCG} ≥ 2 IU/L. Women with LH_{HCG} < 2 IU/L had lower high-quality embryo rate (42.2% *vs.* 46.5%, $p = 0.002$) and implantation rate (40.0% *vs.* 58.8%, $p = 0.044$) compared to those with LH_{HCG} ≥ 2 IU/L. When LH_{HCG} < 2 IU/L, there was no significant difference in implantation rates in patients < 35 years compared to those ≥ 35 years. When LH_{HCG} ≥ 2 IU/L, patients < 35 years old had higher implantation rates (71.7% *vs*. 41.2%, $p < 0.001$) compared to those ≥ 35 years old. The success rates of IVF fertilization and ICSI fertilization and biochemical and clinical pregnancy rates were not significantly different between groups. Our results demonstrated that women undergoing IVF/ICSI-ET might benefit from maintaining LH_{HCG} levels at ≥ 2 IU/L. In addition, age might associate with LH_{HCG} levels and be a better determining factor of the transfer outcome than serum LH_{HCG} levels for IVF/ICSI-ET.

Keywords luteinizing hormone, human chorionic gonadotropin, IVF/ICSI-ET, gonadotropin-releasing hormone antagonist, pregnancy outcomes

1. Introduction

The quality of follicles is different according to various controlled ovarian hyperstimulation (COH) treatments during *in vitro* fertilization/intracytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET), impacting pregnancy outcomes (*1*). Gonadotropin-releasing hormone (GnRH) agonist (GnRH-a) and GnRH antagonist (GnRH-ant) protocol are clinically used to control the quality of follicles. The GnRH-ant can block the GnRH receptor without causing a premature surge in luteinizing hormone (LH), resulting in a shorter period of ovulation induction, less reliance on gonadotropins (Gn), and fewer cases of severe ovarian hyperstimulation syndrome than the GnRH-a long protocol (*2,3*). However, the main drawback of GnRH-ant administration was a rapid and significant reduction of serum LH levels, which adversely affected oocyte retrieval and embryo quality, resulting in poor outcomes in IVF/ICSI-ET (*4*). Appropriate GnRH-ant protocols have become increasingly prevalent in assisted reproductive technology (ART).

LH, a pituitary hormone regulated by GnRH, interacts with its receptors and promotes the maturation of ovarian follicles. A surge of LH triggers ovulation in the middle of the menstrual cycle, which prompts the corpus luteum to produce progesterone, essential for maturing the uterine endometrium to prepare for the implantation of a fertilized egg (*5*). Low endogenous levels of LH adversely affect the development of normal follicles and the endometrium after ovulation (*6*). A study has shown that low serum LH levels the day following a GnRH-a trigger are linked to a decreased rate of ongoing pregnancies, high miscarriages, and low live births (*7*). In women receiving human chorionic gonadotropin (HCG) with less than 30% LH in the serum, the pregnancy and implantation rate was significantly reduced (*7,8*). However, other contrary findings indicated pregnancy outcomes were similar for patients with low and high serum LH levels (*9-11*). Still, it is unclear whether GnRH-ant should be used based on serum LH levels during COH. Despite the fact that supplementing LH during GnRH-ant administration compensates for the severe decrease in levels of endogenous LH caused by the GnRH-ant (*4*), there is controversy whether patients with endogenous LH deficiency can benefit from GnRH-ant treatment.

In addition, age is supposed to be an independent factor affecting serum LH levels and pregnancy outcomes. Clinical studies suggest that LH-supplemented ovarian stimulation was not beneficial to younger women but that women over 35 are likely to benefit from LH combined with follicle-stimulating hormone (FSH) (*12,13*). Whether exogenous LH supplementation and age factor can effectively improve pregnancy outcomes is an unmet clinical question.

The research was conducted to evaluate the impact of LH level on HCG day (LH_{HCG}) and age on outcomes of IVF/ICSI-ET patients using the GnRH-ant protocol. We retrospectively analyzed 620 women who had undergone IVF/ICSI-ET and accepted the GnRH-ant protocol. Exogenous LH was applied if needed during the GnRH-ant protocol. Fertilization and pregnancy outcomes were investigated from the different groups, which were divided according to LH_{HCG} level (2 IU/L) and age (35 years).

2. Patients and Methods

2.1. Patients and study design

This retrospective study was performed in Taizhou Hospital, Zhejiang Province, from January 2018 to December 2020. Inclusion criteria were as follows: *i*) Aged 20-50 years old; *ii*) underwent IVF/ICSI-ET and accepted GnRH-ant protocol. Exclusion criteria included: *i*) Incomplete study data; *ii*) uterine pathology or morphological abnormality; *iii*) those with gonadal disorders or conditions that affected the secretion or excretion of sex hormones and *v*) chromosome abnormality. Ethical approval was obtained by the Ethics Committee of Taizhou Hospital, Zhejiang Province. During the study, 1276 women were screened based on inclusion and exclusion criteria. Stratified analyses were performed with the patient population according to a level of serum LH (LH_{HCG} < 2 IU/L *versus* $LH_{HCG} \geq 2$ IU/L) and age (< 35 years *versus* ≥ 35 years). In total, given the confounding factors of frozen embryo implantation, only 125 women with fresh embryo implantation were eligible for implantation analysis (Figure 1).

2.2. GnRH-ant protocol

Gn was intramuscularly injected to induce ovulation if serum basal levels of FSH and LH conformed to standard on the 2nd-4th day of menstruation, and no functional follicles were showed by transvaginal ultrasound. The initial gonadotropin dose of Gn (Lishenbao, 75 U, Lizhu Group) varied from 150 to 225 IU according to serum hormone levels and antral follicle count (AFC). During COH, the dose for GnRH-antagonist (Sizekai, 0.25 mg, Merck Cerano, France) injection depended on the follicle's diameter (≥ 14 mm) and serum basal LH levels (> 10 IU/L). Follicular development was monitored on vaginal ultrasound examination and hormone levels. When serum LH level is too low and follicular development is not synchronized, human menopausal gonadotropin (HMG, Lebaode, 75 IU, Lizhu Group, Zhuhai, China) is injected to supplement exogenous LH. When ≥ 3 follicles reached a diameter of ≥ 17 mm, or ≥ 2 follicles reached a diameter of ≥ 18 mm, 5000-10000 IU of HCG was administered. Aspiration of the oocytes was carried out 36 hours after injection of HCG with ultrasound guidance.

2.3. IVF/ICSI-ET

IVF was performed as a routine, while ICSI was conducted in the following cases: *i*) Patients with previous conventional fertilization failure or fertilization rate < 30%; *ii*) spouses with severe oligospermia, asthenospermia, and teratozoospermia; *iii*) obstructive, non-obstructive azoospermia; *iv*) difficult to take sperm or unable to ejaculate after taking eggs; *vi*) cryopreservation of a limited number of sperm. Normal fertilization could be confirmed when two pronuclei (2PN) were visible 16-18 h after insemination. Three days after oocyte retrieval, the decision to perform a transplant was made in accordance with embryo grading, hormone levels, and the condition of the endometrium. A suitable number of embryos was transferred according to Chinese legislation: For the first time in their reproductive years, women under 35 can only be transferred two embryos, whereas women

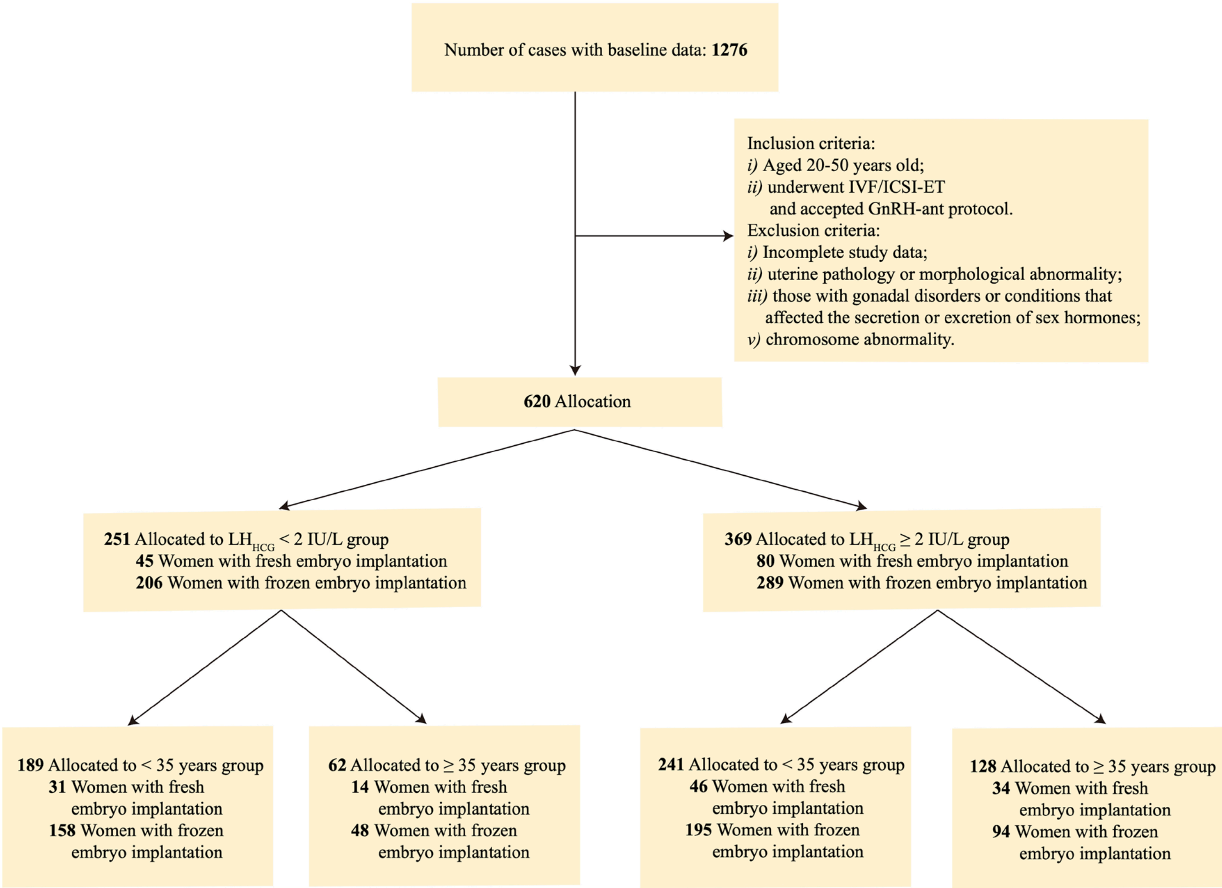

Figure 1. Flow chart of patient disposition

over 35 or who have failed IVF/ICSI-ET several times can receive two to three embryos (*14*). The high-quality blastocysts were freshly transplanted or cryopreserved, and the non-high-quality blastocysts were discarded.

2.4. Outcomes

There were two main outcomes: biochemical and clinical pregnancy rates. Biochemical pregnancy was confirmed as serum β-HCG > 25 IU/L within 12-14 days of embryo transfer (*15*). Clinical pregnancy was confirmed according to gynecological ultrasonography 30-35 days after embryo transfer (*16*). Furthermore, we evaluated the implantation rate, high-quality embryo rate, normal fertilization rate of IVF, and normal fertilization rate of ICSI. Age, body mass index (BMI), infertility duration, bilateral AFC, basal FSH level, basal LH level, basal FSH/LH, basal estradiol, LH_{HCG}, progesterone level on HCG day (P_{HCG}), amount of follicles ≥ 14 mm on HCG day, retrieved oocyte number, mature oocyte number, 2PN embryo count, and high-quality embryo count were also analyzed.

2.5. Statistical methods

In normal distributions, the mean and standard deviation are used to represent continuous variables. In view of the non-normal distribution of the data, continuous variables have been expressed as median (quartile range). When analyzing continuous variables, the Mann-Whitney *U* test was used. With categorical variables expressed as percentages, the Chi-squared test or Fisher's exact probability were used to test the data. Statistical significance was reached at $p < 0.05$, and statistical analyses were conducted with SPSS 25.0 (IBM, Chicago, IL, USA).

3. Results

3.1. Participant characteristics

All 620 patients were divided into LH_{HCG} < 2 IU/L group and LH_{HCG} ≥ 2 IU/L group based on serum LH_{HCG}. There were no differences in BMI (p = 0.076) and infertility duration (p = 0.451) between the two groups. However, a younger age (p = 0.006) was observed in the LH_{HCG} < 2 IU/L group. No significant differences were found in infertility duration, bilateral AFC, basal estradiol, and P_{HCG} between the two groups. Patients in the LH_{HCG} < 2 IU/L group had lower basal FSH levels (7.4 *vs.* 7.8, p = 0.006), lower basal LH levels (4.0 *vs.* 4.7, p < 0.001), and greater basal FSH/LH (1.83 *vs.* 1.73, p = 0.049) (Table 1). Thus, age might associate with LH_{HCG} levels.

3.2. Patients in LH_{HCG} < 2 IU/L group trended to be fertilized but had a lower implantation rate

A suitable range of LH levels for achieving pregnancy has not been established. Some studies conclude LH_{HCG} may predict the fate of IVF, in which LH = 2 IU/L is an important demarcation point (*17,18*). In this study, we investigated the outcomes of fertilization and implantation according to the level of LH. The embryo transfer rate was no significant difference between LH_{HCG} < 2 IU/L group (45/251, 17.9%) and LH_{HCG} ≥ 2 IU/L group (80/369, 21.7%). A comparison of LH_{HCG} groups was carried out to examine fertility and pregnancy outcomes. Compared to LH_{HCG} ≥ 2 IU/L group, patients in the LH_{HCG} < 2 IU/L group had more number of follicles ≥ 14 mm on HCG day, retrieved oocytes, mature oocytes, and 2PN embryos, but lower high-quality embryo rate (42.2% *vs.* 46.5%, $p = 0.002$) and implantation rate (40.0% *vs.* 58.8%, $p = 0.044$). The median number of high-quality embryos (3 *vs.* 2, $p = 0.076$), IVF fertilization rate (75.9% *vs.* 75.5%, $p = 0.710$), ICSI fertilization rate (84.0% *vs.* 83.8%, $p = 0.874$), biochemical pregnancy (17.8% *vs.* 15.0%, $p = 0.684$) rate and clinical pregnancy rate (31.1% *vs.* 43.8%, $p = 0.165$) were no significant differences between two groups (Table 2). Therefore, patients with LH_{HCG} < 2 IU/L intended to be fertilized, while patients with LH_{HCG} ≥ 2 IU/L had a higher implantation rate.

3.3. Subgroup analysis

3.3.1. Participant characteristics in different subgroups

A woman's LH bioactivity typically falls across the threshold of 35 years (*19*). LH_{HCG} < 2 IU/L group and LH_{HCG} ≥ 2 IU/L group were further classified according to age. When LH_{HCG} < 2 IU/L, no difference was observed regarding BMI, infertility duration, basal FSH levels, basal estradiol, and P_{HCG} in different ages; patients in the < 35 years group had more bilateral AFC (15.0 *vs.* 8.5, $p < 0.001$), higher basal LH levels (4.10 *vs.* 3.70, $p = 0.009$), and lower basal FSH/LH (1.76 *vs.* 2.18, $p = 0.006$) than those ≥ 35 years (Table 3). When LH_{HCG} ≥ 2 IU/L, the groups did not differ significantly in BMI and infertility duration in the < 35 years group and ≥ 35 years group. Meanwhile, patients in the < 35 years group had more bilateral AFC (14 *vs.* 9, $p < 0.001$), lower basal FSH levels and FSH/LH ratio, increased basal LH levels (5.20 *vs.* 4.20, $p < 0.001$), higher basal estradiol levels (41.00 *vs.* 36.50, $p = 0.031$), and increased P_{HCG} levels (1.01 *vs.* 0.84, $p < 0.001$) in the LH_{HCG} ≥ 2 IU/L group (Table 4).

Table 1. Characteristics and ovarian reserve of patients with different LH_{HCG} levels

Variables	LH_{HCG} < 2 IU/L	LH_{HCG} ≥ 2 IU/L	*p*
Age (years)	31 (28-34)	32 (29-36)	0.006
BMI (kg/m^2)	22.0 (20.0-24.3)	22.6 (20.3-25.1)	0.076
Infertility duration (years)	3.0 (2.0-5.0)	2.5 (2.0-5.0)	0.451
Bilateral AFC	13 (9-19)	12 (8-19)	0.072
Basal FSH (IU/L)	7.4 (6.3-8.6)	7.8 (6.5-9.4)	0.006
Basal LH (IU/L)	4.0 (3.0-5.4)	4.7 (3.5-6.4)	< 0.001
Basal FSH/LH	1.83 (1.34-2.53)	1.73 (1.18-2.33)	0.049
Basal estradiol (pg/mL)	37.0 (27.0-54.0)	39.2 (27.0-52.5)	0.678
P_{HCG} (ng/mL)	1.06 (0.73-1.47)	0.97 (0.66-1.40)	0.189

LH: luteinizing hormone; HCG: human chorionic gonadotropin; LH_{HCG}: LH levels on HCG day; BMI: body mass index; AFC: antral follicle counting; FSH: follicle stimulating hormone; P_{HCG}: progesterone levels on HCG day.

Table 2. Embryological and pregnancy outcomes of patients with different LH_{HCG} levels

Variables	LH_{HCG} < 2 IU/L	LH_{HCG} ≥ 2 IU/L	*p*
Embryo transfers (*n*, %)	45/251 (17.9)	80/369 (21.7)	0.264
Number of follicles ≥ 14 mm on HCG day	9.0 (6.0-13.0)	7.0 (4.0-11.5)	< 0.001
Number of oocytes obtained	10.0 (6.0-15.0)	8.0 (5.0-13.0)	< 0.001
Number of mature oocytes	10.0 (6.0-14.0)	7.0 (4.5-12.0)	< 0.001
Number of 2PN embryos	8 (5-12)	6 (3-10)	< 0.001
Normal fertilization rate of IVF (*n*, %)	2237/2949 (75.9)	2678/3549 (75.5)	0.710
Normal fertilization rate of ICSI (*n*, %)	2237/2664 (84.0)	2678/3195 (83.8)	0.874
Number of high-quality embryos	3 (1-5)	2 (1-5)	0.076
High-quality embryo rate (*n*, %)	944/2237 (42.2)	1246/2678 (46.5)	0.002
Implantation rate (*n*, %)	18/45 (40.0)	47/80 (58.8)	0.044
Biochemical pregnancy rate (*n*, %)	8/45 (17.8)	12/80 (15.0)	0.684
Clinical pregnancy rate (*n*, %)	14/45 (31.1)	35/80 (43.8)	0.165

LH: luteinizing hormone; HCG: human chorionic gonadotropin; LH_{HCG}: LH levels on HCG day; 2PN: two pronuclei; IVF: *in vitro* fertilization; ICSI: intracytoplasmic sperm injection.

Table 3. Patients characteristics in the LH_{HCG} < 2 IU/L group

Variables	LH_{HCG} < 2 IU/L		*p*
	< 35 years	≥ 35 years	
Age (years)	30 (27-32)	37 (36-40)	< 0.001
BMI (kg/m^2)	22.03 (19.53-24.37)	21.85 (20.65-24.30)	0.450
Infertility duration (years)	3.0 (2.0-5.0)	3.0 (1.0-6.3)	0.774
Bilateral AFC	15.0 (10.0-20.0)	8.5 (6.0-12.3)	< 0.001
Basal FSH (IU/L)	7.30 (6.25-8.50)	7.50 (6.68-8.90)	0.394
Basal LH (IU/L)	4.10 (3.20-5.50)	3.70 (2.60-4.45)	0.009
Basal FSH/LH	1.76 (1.30-2.31)	2.18 (1.50-2.90)	0.006
Basal estradiol (pg/mL)	36.00 (27.00-53.35)	44.00 (26.75-54.75)	0.464
P_{HCG} (ng/mL)	1.09 (0.77-1.54)	0.95 (0.55-1.36)	0.096

LH: luteinizing hormone; HCG: human chorionic gonadotropin; LH_{HCG}: LH levels on HCG day; BMI: body mass index; AFC: antral follicle counting; FSH: follicle stimulating hormone; P_{HCG}: progesterone levels on HCG day.

Table 4. Patients characteristics in the LH_{HCG} ≥ 2 IU/L group

Variables	LH_{HCG} ≥ 2 IU/L		*p*
	< 35 years	≥ 35 years	
Age (years)	30 (27-32)	37 (36-40)	< 0.001
BMI (kg/m^2)	22.70 (20.00-25.10)	22.50 (20.55-25.00)	0.766
Infertility duration (years)	3 (2-5)	2 (1-6)	0.264
Bilateral AFC	14 (10-20)	9 (6-12)	< 0.001
Basal FSH (IU/L)	7.40 (6.30-8.85)	8.60 (7.23-10.28)	< 0.001
Basal LH (IU/L)	5.20 (3.60-7.15)	4.20 (3.33-5.30)	< 0.001
Basal FSH/LH	1.44 (1.00-2.14)	2.05 (1.65-2.78)	< 0.001
Basal estradiol (pg/mL)	41.00 (29.00-54.00)	36.50 (24.00-50.68)	0.031
P_{HCG} (ng/mL)	1.01 (0.73-1.54)	0.84 (0.53-1.26)	< 0.001

LH: luteinizing hormone; HCG: human chorionic gonadotropin; LH_{HCG}: LH levels on HCG day; BMI: body mass index; AFC: antral follicle counting; FSH: follicle stimulating hormone; P_{HCG}: progesterone levels on HCG day.

3.3.2. When LH_{HCG} ≥ 2 IU/L, patients < 35 years had higher implantation rates than those ≥ 35 years

Better implantation and pregnancy rates have been reported when patients aged ≥ 35 years had LH supplementation added to COH protocol, while others disagree (*20,21*). In this study, subgroup-wise differences analyses were performed to investigate whether adding exogenous LH in women ≥ 35 years benefits under the IVF/ICSI-ET. The results revealed that the number of follicles ≥ 14 mm on HCG day, retrieved oocytes, mature oocytes, and 2PN embryos were significantly higher in the < 35 years group than those in the ≥ 35 years group, regardless of whether LH_{HCG} < 2 IU/L (Table 5) or LH_{HCG} ≥ 2 IU/L (Table 6). Statistics did not show any significant differences in embryo transfer rate, normal fertilization rate on IVF or ICSI, high-quality embryos rate, biochemical pregnancy rate, and clinical pregnancy rate between the < 35 years group and ≥ 35 years group, no matter whether LH_{HCG} < 2 IU/L (Table 5) or LH_{HCG} ≥ 2 IU/L (Table 6). But when LH_{HCG} ≥ 2 IU/L, patients < 35 years old had higher implantation rates (71.7% *vs.* 41.2%, $p = 0.006$) (Table 6). Maintaining serum LH_{HCG} levels in the range of ≥ 2 IU/L may benefit women under the IVF/ICSI-ET and GnRH-ant protocol. In addition, age may be a better determining factor of the transfer outcome than serum LH_{HCG} levels for IVF/ICSI-ET.

4. Discussion

In this study, serum LH_{HCG} levels were analyzed to determine if they affected outcomes in IVF/ICSI-ET. The results revealed that patients with LH_{HCG} ≥ 2 IU/L had better ovarian reserve capacity, a higher rate of high-quality embryos, and a higher implantation rate. Among patients with low and high levels of LH_{HCG}, there were no statistical differences with regard to clinical outcomes, including fertilization rates in IVF, fertilization rates in ICSI, number of high-quality embryos, biochemical pregnancy rates, and clinical pregnancy rates, in line with previous findings (*9-11,22,23*).

In IVF, GnRH-ant protocols are increasingly preferred, compared to GnRH-a long protocols, because they align with physiological processes (*2*). Women undergoing an IVF/ICSI-ET program are routinely given GnRH-ant to prevent premature LH surges (*24*). Through competitively blocking the GnRH receptor, GnRH-ant administration could rapidly suppress pituitary LH secretion (*25*). Regulating follicle growth and ovulation by FSH and LH is

Table 5. Embryological and pregnancy outcomes of patients in the LH_{HCG} < 2 IU/L group

Variables	LH_{HCG} < 2 IU/L		*p*
	< 35 years	≥ 35 years	
Embryo transfers (*n*, %)	31/189 (16.4)	14/62 (22.6)	0.340
Number of follicles ≥ 14 mm on HCG day	11.0 (7.0-14.0)	6.5 (4.8-9.0)	< 0.001
Number of oocytes obtained	11.0 (9.0-16.0)	7.0 (4.0-10.3)	< 0.001
Number of mature oocytes	10 (7-15)	6 (4-9)	< 0.001
Number of 2PN embryos	9 (5-13)	6 (3-8)	< 0.001
Normal fertilization rate of IVF (*n*, %)	1851/2444 (75.7)	386/505 (76.4)	0.738
Normal fertilization rate of ICSI (*n*, %)	1851/2206 (83.9)	386/458 (84.3)	0.843
Number of high-quality embryos	4 (2-6)	2 (1-4)	0.009
High-quality embryo rate (*n*, %)	766/1851 (41.4)	178/386 (46.1)	0.087
Implantation rate (*n*, %)	15/31 (48.4)	3/14 (21.4)	0.087
Biochemical pregnancy rate (*n*, %)	6/31 (19.4)	2/14 (14.3)	0.681
Clinical pregnancy rate (*n*, %)	12/31 (38.7)	2/14 (14.3)	0.101

LH: luteinizing hormone; HCG: human chorionic gonadotropin; LH_{HCG}: LH levels on HCG day; 2PN: two pronuclei; IVF: *in vitro* fertilization; ICSI: intracytoplasmic sperm injection.

Table 6. Embryological and pregnancy outcomes of patients in the LH_{HCG} ≥ 2 IU/L group

Variables	LH_{HCG} ≥ 2 IU/L		*p*
	< 35 years	≥ 35 years	
Embryo transfers (*n*, %)	46/241 (19.1)	34/128 (26.6)	0.111
Number of follicles ≥ 14 mm on HCG day	8 (5-13)	5 (3-7)	< 0.001
Number of oocytes obtained	10 (7-15)	5 (3-8)	< 0.001
Number of mature oocytes	9 (6-14)	5 (3-7)	< 0.001
Number of 2PN embryos	8 (5-12)	4 (2-6)	< 0.001
Normal fertilization rate of IVF (*n*, %)	2089/2769 (75.4)	589/780 (75.5)	0.968
Normal fertilization rate of ICSI (*n*, %)	2089/2481 (84.2)	589/714 (82.5)	0.275
Number of high-quality embryos	3 (2-6)	2 (1-3)	< 0.001
High-quality embryo rate (*n*, %)	965/2089 (46.2)	281/589 (47.7)	0.515
Implantation rate (*n*, %)	33/46 (71.7)	14/34 (41.2)	0.006
Biochemical pregnancy rate (*n*, %)	7/46 (15.2)	5/34 (14.7)	0.949
Clinical pregnancy rate (*n*, %)	24/46 (52.2)	11/34 (32.4)	0.077

LH: luteinizing hormone; HCG: human chorionic gonadotropin; LH_{HCG}: LH levels on HCG day; 2PN: two pronuclei; IVF: *in vitro* fertilization; ICSI: intracytoplasmic sperm injection.

one of the key factors to improve the success rate of ART. Studies have indicated that FSH and LH surges promote extracellular matrix synthesis and expansion in cumulus cells, contributing to oocyte meiosis. This is crucial for oocyte maturation, ovulation, fertilization, and early embryonic development (*26,27*). A serum LH threshold of < 0.5 IU/L has been proposed as a diagnostic criterion for severe luteinizing depression after IVF ovarian stimulation (*28*). Clinical studies have shown that LH levels below 0.5 IU/L in GnRH-ant treatment significantly lower the rate of implantation and pregnancy. For patients with low levels of LH, adding low-dose urinary HCG improves implantation and live birth rates (*29*). An adequate serum LH level of 1.2 IU/L is required to support FSH-induced follicular development (*30,31*). Thus, adjusting LH levels is of great significance for protecting embryo development in ART. However, relevant studies are disputable about whether endogenous LH should be replenished and which is a suitable level of LH_{HCG} (*32*). In this study, results demonstrated that patients with LH_{HCG} ≥ 2 IU/L had higher implantation rates than those with LH_{HCG} < 2 IU/L when patients ≥ 35 years; therefore, maintaining LH_{HCG} levels in the range of ≥ 2 IU/L can be suitable, especially in women ≥ 35 years.

FSH/LH ratio and bilateral AFC reflect ovarian reserve, which can predict ovarian reserve degradation and COH response (*33*). Studies have shown that the ratio of FSH/LH is positively correlated with age (*34*). It is consistent with our subgroup analysis result that women aged < 35 years had lower FSH/LH and higher bilateral AFC, indicating that patients aged < 35 years had better ovarian reserve capacity. Our study also observed that the basal FSH/LH in LH_{HCG} < 2 IU/L group was higher than that in the LH_{HCG} ≥ 2 IU/L group, which means that patients with LH_{HCG} ≥ 2 IU/L have a better ovarian reserve.

The quality of follicles obtained by hyperstimulation is far more important than the number of follicles. A complex intrafollicular process that controls oocyte maturation affects oocyte quality and impedes embryo implantation (*35*). ARTs have been challenged by the need to select embryos with higher implantation potential (*36*). Our study indicated that high LH levels are not conducive to follicle excretion and maturation in the early stages of ovulation stimulation, but $LH_{HCG} \geq 2$ IU/L is associated with high-quality embryos and implantation. Previous studies have shown that supplementing LH activity in the mid-follicle produces favorable pregnancy outcomes in low-responders (*37*). LH activity administered only in low doses of HCG can support the final stages of follicular development without causing premature luteinization, which supports our theory (*38*).

Increased serum estradiol levels during ovarian hyperstimulation can be good predictive properties for IVF-ET outcomes (*39*). Although there is no direct association between P_{HCG} and clinical pregnancy among women undergoing ovarian stimulation with gonadotropins (*40*), progesterone can cause endometrial thickening, congestion, gland hyperplasia, and branching after ovulation, which is conducive to pregnancy and embryo development. This study showed that estradiol levels remained high during ovarian stimulation, and no significant difference was found between estradiol and P_{HCG} levels according to LH_{HCG} levels. However, when $LH_{HCG} \geq 2$ IU/L, patients with age < 35 had higher levels of estradiol and P_{HCG}, suggesting that $LH_{HCG} \geq 2$ IU/L and age < 35 can be used as an indication for IVF/ICSI-ET outcomes.

It can be concluded that with age, the quantity and quality of follicles obtained by superovulation stimulation and the quality of embryos gradually decrease, consistent with previous studies (*41,42*). Notably, when patients' $LH_{HCG} < 2$ IU/L, no difference was observed in implantation rate among different age groups, while when patients' $LH_{HCG} \geq 2$ IU/L, patients < 35 years had a higher implantation rate. The result suggests that patients aged ≥ 35 years are more likely to require serum LH_{HCG} levels in the range of ≥ 2 IU/L to meet the needs of embryo transfer and later development.

In conclusion, it is meaningful to monitor and control LH_{HCG} levels to improve ovarian reserve function and blastocyst implantation after transplantation during GnRH-ant protocols because of higher rates of high-quality embryos and implantation. Exogenous LH supplementation may be efficacious in improving pregnancy outcomes in this study; still, the specific level of LH_{HCG} needs further prospective research.

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