Is it necessary to monitor the serum luteinizing hormone(LH) concentration on the human chorionic gonadotropin (HCG) day among young women during the follicular-phase long protocol? —A retrospective cohort study

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Abstract

Background

The normal physiological function of LH requires a certain concentration range, but because of pituitary desensitization, even on the HCG day, endogenous levels of LH are low in the follicular-phase long protocol. So our study aimed to determine whether it is necessary to monitor serum LH concentrations and to determine whether there is an optimal LH range to achieve the desired clinical outcome.

Methods

A retrospective cohort study included 4503 cycles of in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) units from January 1, 2016, to June 30, 2019, in a single department. The main outcome measures included retrieved eggs, available embryos, live birth rate.

Results

Divided the LH_{HCG} into five groups: Group A ($LH \leq 0.5$), Group B (0.5 IU/L $< LH \leq 1.2$ IU/L), Group C (1.2 IU/L $< LH \leq 2.0$ IU/L), Group D (2.0 IU/L $< LH \leq 5.0$ IU/L), Group E ($LH > 5$ IU/L). In terms of the numbers of retrieved eggs, embryos, high-quality embryos and diploid fertilized oocytes, an increase of the LH_{HCG} level showed a trend of a gradual decrease. However, there was no significant difference in clinical outcomes among the groups.By adjusting for confounding factors, with an increase in LH_{HCG}, the number of retrieved eggs decreased.

Conclusion

In the follicular-phase long protocol among young women, monitoring of LH_{HCG} are recommended in the clinical guidelines. What's more, those who undergo Preimplantation Genetic Testing(PGT) may benefit more when the LH level is controlled within a certain range.

Introduction

Appropriate protocols of controlled ovarian stimulation (COS) are critical for assisted reproductive technology (ART) outcomes. The discovery of gonadotropin-releasing hormone (GnRH) analogs has offered multiple options in assisted reproduction and improved in vitro fertilization (IVF) success rates (1). Effective control of the premature luteinizing hormone (LH) peak, reduction of the cycle cancellation rate and more mature oocytes make the GnRH agonist protocol popular in many reproductive centers. In China, the follicular-phase long protocol has gradually become mainstream because of its simple and convenient single administration, good follicular homogeneity, and excellent fresh cycle outcomes (2). In

the GnRH agonist protocol, because of pituitary desensitization, endogenous levels of LH are very low during the late stimulation phase (3). Moreover, approximately 50% of patients undergoing IVF/intracytoplasmic sperm injection (ICSI) using a GnRH agonist are LH deficient (3). Thus, it would seem logical that LH supplementation would be beneficial.

According to the “two-cell, two-gonadotrophin theory”, both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are important for follicle development in humans. This model explains our understanding of folliculogenesis (4). LH stimulates theca cells, promoting androgen production, and FSH regulates the proliferation of granulosa cells (GCs) and promotes E2 synthesis. Whereas FSH is the main regulator of antral follicular growth, LH plays key roles in promoting steroidogenesis and in the development of the leading follicle. Moreover, LH exerts different functions during the different stages of both natural and stimulated cycles (5). During the menstrual cycle, LH not only promotes the growth of larger follicles but also increases granulosa cell FSH activity by increasing androgen synthesis, and then LH promotes the recruitment of follicles.

The normal physiological function of LH requires a certain concentration range, namely, the “LH window” (6). When below the LH threshold, the eggs cannot fully mature, and there is not enough androgen and estrogen synthesis. Moreover, there is a lack of paracrine signals between granulosa cells and membrane cells. In contrast, when beyond the upper limit, granulosa cell proliferation is inhibited, which can lead to a series of problems in egg development. Therefore, we speculate that it is necessary to monitor the level of LH during the early follicular phase long protocol.

An increasing amount of evidence has demonstrated that downregulation with a GnRH agonist in some normogonadotrophic women may result in profound suppression of LH, which in turn impairs adequate estradiol synthesis during FSH stimulation for IVF/ICSI (4–7), and reduces the fertilization rate (8), the number of clinical pregnancies (8), and the pregnancy outcomes (9). However, there is some controversy about this conclusion.

Consequently, this study aimed to determine whether it is necessary to monitor the serum LH concentration on the HCG day(LH_{HCG}), to identify whether the LH level on the human chorionic gonadotropin (HCG) day (LH_{HCG}), have an impact on the clinical outcome and to determine whether there is an optimal LH range to achieve the expected clinical outcome.

Material And Methods

Study design and participants

The subjects of this retrospective study underwent 4503 cycles of IVF or ICSI units from January 1, 2016 to June 30, 2019 in our department. The following inclusion criteria were applied: 1) age ≤ 40 years, 2) follicular-phase single-dose GnRH agonist protocol, 3) fresh cycle transplants, HCG endometrial thinning, recurrent miscarriage, and endometriosis.

The study was approved by the ethics committee of the Third Affiliated Hospital of Zhengzhou University(2021 – 105).

Data on patient age and infertility treatment-related characteristics were collected from the files. Basic sex hormone levels (follicle stimulating hormone [FSH], luteinizing hormone [LH], estradiol [E2], progesterone [P]), Gn dose, duration of Gn stimulation, number of oocytes obtained, number of oocytes retrieved, the two pronuclear zygote (2PN) embryo rate, the high-quality embryo rate, the number of embryos transferred, and the LH level on day1 of ovarian stimulation/the HCG day were recorded. E2/P/on the day of hCG administration was recorded. Clinical pregnancy was defined as visualization of both a gestational sac and fetal cardiac activity on transvaginal ultrasound. Early spontaneous abortion refers to pregnancy failure that occurred before 12 weeks.

Stimulation protocols

Follicular-phase single-dose GnRH agonist protocol: A single dose of 3.75 mg long-acting GnRH agonist triptorelin (Diphereline, IPSEN, Paris, France) was administered on day 2 or 3 of the menstrual cycle. Twenty-eight days later, serum FSH, LH, E2, and P were examined. When FSH and LH were both < 5 IU/L, P < 1 μg/L and E2 < 50 pg/ml, gonadotropin (Gn) was initiated daily until ovulation induction. HMG (Livzon, China, Zhuhai) or recombinant LH (rLH Luveris Merck Serono S. A, Beijing) was added according to follicular development. When three follicles reached a mean diameter of 17 mm or two follicles reached a mean diameter of 18 mm, 0.25 mg of recombinant LH (Ovidrel, Merck Serono S.A., Beijing) was administered intramuscularly. Oocyte retrieval was performed 36 h after hCG injection by transvaginal ultrasound-guided single-lumen/double-lumen needle aspiration. Intracytoplasmic sperm injection (ICSI) was performed only for severe male factor infertility or previous fertilization failure. Luteal phase support was initiated on day1 after oocyte retrieval. The cleavage stage embryo transfer time was 3 days after egg retrieval. The blastocyst transfer time was 5 days after egg retrieval. Morphologic criteria were used for embryo scoring (10).

Statistical analysis

Differences in variables between the groups were statistically analyzed with Student's t-test and chi-squared tests when appropriate. A bilateral p value < 0.05 was considered to be significant. The results are presented as the mean ± standard deviation. If the variances were not uniform, we used the rank sum test for comparison.

Linear regression analysis was performed to assess the association between the LH level on the HCG day and the number of eggs retrieved, whereas logistic regression analysis was used to evaluate the correlation with the rate of conception. The same set of potential confounders was introduced into the regression models for adjustment by the enter method, regardless of whether significant differences between groups were observed. Statistical analysis was performed with the Statistical Package for the Social Sciences (version 24.0; SPSS Inc, USA). All P < 0.05 on one-sided tests was considered to be statistically significant.

Results

The influence of LH_{HCG} level on the outcome of IVF/ICSI-ET

When the serum LH level is lower than 0.5 IU/L, both the fertilization rate and the number of embryos decreases significantly. Some clinicians believe that the threshold of LH can be set to 1.2 IU/L; if the LH is lower than this level, follicular development and endometrial growth will be severely insufficient (11). When the serum LH level is greater than 5.0 IU/L on the day of hCG in the follicular-phase long protocol, the pregnancy rate significantly decreased (12). Thus, we divided the LH_{HCG} into five groups: Group A ($LH \leq 0.5$), Group B ($0.5\ IU/L < LH \leq 1.2\ IU/L$), Group C ($1.2\ IU/L < LH \leq 2.0\ IU/L$), Group D ($2.0\ IU/L < LH \leq 5.0\ IU/L$), Group E ($LH > 5\ IU/L$).

There were 9 cycles with $LH_{HCG} \geq 10$ IU/L in the follicular-phase long protocol, of which only 1 cycle was greater than 20 IU/L. There was no obvious follicle luteinization in these cycles.

According to the basic information and clinical characteristics of the patients, the age of group D was slightly lower than that of group C. The BMI of groups D and E was lower. The basal FSH and LH in groups C, D and E were higher than those in groups A and B (Table 1).

Table 1
Patient characteristics of clinical

	Group A(LH ≤ 0.5)	Group B (0.5 LH ≤ 1.2)	Group C (1.2 LH ≤ 2)	Group D (2 LH ≤ 5)	Group E (LH 5)	*P*
No.of cycles	225	2143	1495	580	60	
Age of woman(years)	29.80 ± 3.93	29.69 ± 3.89	29.92 ± 3.82	29.30 ± 3.80[c]	29.65 ± 3.92	0.025
Body mass index(kg/m^2)	23.91 ± 3.50	23.80 ± 3.22	23.56 ± 3.17	22.49 ± 3.18[abc]	22.52 ± 3.37[ab]	0.000
Basic hormone concentrations						
FSH(IU/L)	6.38 ± 1.86	6.64 ± 1.90	7.06 ± 2.12[ab]	7.35 ± 2.13[ab]	7.73 ± 2.33[ab]	0.000
LH(IU/L)	4.89 ± 3.60	5.38 ± 3.77	5.99 ± 3.90[ab]	6.80 ± 5.67[abc]	6.71 ± 4.81[ab]	0.000
P(nmol/L)	145.60 ± 64.69	152.96 ± 109.13	155.76 ± 146.55	159.43 ± 169.57	164.23 ± 120.90	0.615
E2(pmol/L)	1.52 ± 2.17	1.42 ± 1.79	1.45 ± 3.03[ab]	1.57 ± 3.65	1.90 ± 2.63	0.000
AMH(pmol/L)	37.00 ± 23.12	33.16 ± 22.03	31.90 ± 22.52[a]	32.82 ± 23.50	32.98 ± 23.32	0.029
Start-up Gn does(IU)	163.90 ± 52.36	167.20 ± 56.47	169.17 ± 60.02	157.46 ± 58.74[bc]	157.71 ± 62.91	0.000
Total Gn dose (IU)	2532.36 ± 993.04	2479.22 ± 1012.23	2529.74 ± 1080.28	2351.01 ± 1083.11[abc]	2308.48 ± 1117.57	0.000
$LH_{day\ 1}$(IU/L)	0.59 ± 0.29	0.72 ± 0.38a	0.97 ± 1.01[ab]	1.06 ± 0.73[abc]	1.05 ± 0.69[ab]	0.000
Hormone concentrations on HCG						

[a]Compared with group A, *P* 0.05

[b]Compared with group B, *P* 0.05

[c]Compared with group C, *P* 0.05

	Group A(LH ≤ 0.5)	Group B (0.5＜LH ≤ 1.2)	Group C (1.2＜LH ≤ 2)	Group D (2＜LH ≤ 5)	Group E (LH＞5)	*P*
E2(pmol/L)	1367.07 ± 6341.47	12884.09 ± 5950.26[ad]	13131.38 ± 5834.56[ad]	14517.02 ± 6740.33[bc]	13151.15 ± 6015.93	0.000
P(nmol/L)	3.89 ± 1.42[bcd]	3.56 ± 1.44[cd]	3.35 ± 1.49	3.21 ± 1.42	3.30 ± 1.34	0.000
Endometrial thickness on HCG	11.35 ± 6.85	11.29 ± 2.26	11.41 ± 2.29[ae]	11.40 ± 2.09	10.56 ± 2.01	0.002
[a]Compared with group A, *P*＜0.05						
[b]Compared with group B, *P*＜0.05						
[c]Compared with group C, *P*＜0.05						

The level of P in groups A and B was higher than that in the other groups. The AMH value of group C was significantly lower than that of group A. The start-up Gn dose in group D was significantly lower than that in groups B and C. The total Gn dose in group D was significantly lower than that in the other groups. For the hormone concentrations of HCG, the E_2 level of group B and group C was lower, and the P level in group A was the highest. On HCG day, the endometrium in group C was thicker than that in groups A and E (Table 1).

In terms of the numbers of retrieved eggs, embryos, high-quality embryos and diploid fertilized oocytes, an increase of the LH_{HCG} level showed a trend of a gradual decrease. Group E had the highest rate of embryo transfer at the cleavage stage. However, there was no significant difference in clinical outcomes among the groups (Table 2).

Table 2
Embryological and clinical outcomes

	Group A(LH ≤ 0.5)	Group B (0.5＜LH ≤ 1.2)	Group C (1.2＜LH ≤ 2)	Group D (2＜LH ≤ 5)	Group E (LH＞5)	*P*
No.of oocytes	15.22 ± 5.66	13.54 ± 5.23[a]	12.90 ± 5.05[ab]	12.30 ± 4.88[ab]	9.6 ± 4.09[abcd]	0.000
No.of diploid fertilized oocytes	9.85 ± 4.70	8.69 ± 4.41[a]	8.39 ± 4.33[a]	7.78 ± 3.96[ab]	5.92 ± 2.78[abcd]	0.000
No.of embryos	7.90 ± 4.48	6.83 ± 4.03[a]	6.44 ± 3.88[ab]	6.22 ± 3.62[ab]	4.40 ± 2.55[abcd]	0.000
No.of high quality embryos	4.32 ± 3.71	3.97 ± 3.42	3.76 ± 3.19	3.71 ± 3.04	2.52 ± 2.27[abcd]	0.007
No.of embryos transferred	1.58 ± 0.49	1.63 ± 0.48	1.65 ± 0.48	1.66 ± 0.48	1.70 ± 0.46	0.203
Types of embryos transferred						
Cleavage stage embryo rate	62.22%	68.60%	70.03%	70.34%	88.33%[abcd]	0.002
Blastocysts rate	37.78%	31.40%	29.97%	29.66%	11.67%	
OHSS rate	3.56%	2.80%	3.95%	4.66%	6.67%	0.090
Biochemical pregnancy rate	69.33%	67.48%	67.56%	68.62%	66.67%	0.964
Clinical pregnancy rate	66.67%	64.35%	63.21%	64.48%	63.33%	0.867
Ectopic pregnancy rate	2.67%	1.16%	1.59%	1.07%	0	0.484
Spontaneous abortion rate	10.67%	9.64%	8.47%	9.09%	2.63%	0.509
Live birth rate	56.44%	55.58%	54.65%	56.03%	60%	0.898
Premature birth rate	21.26%	15.70%	14.44%	17.54%	22.22%	0.216

Logistic regression analysis was used to determine whether the LH_{HCG} level was related to the number of oocytes retrieved. The results showed that in the follicular-phase long protocol, with the increase in LH_{HCG}, the number of retrieved eggs decreased (Table 3 and Fig. 1). In the follicular-phase long protocol, BMI and AMH were not related to the number of eggs retrieved.

Table 3
Linear regression analysis between serum LH concentration of HCG day and eggs retrieved

	Adjusted OR	95% CI	*P*
LH_{HCG}	-0.264	0.939–1.065	0.000
Age of woman(years)	-0.041	0.954–1.049	0.006
BMI	-0.034	0.937–1.067	0.060
Basic LH (IU/L)	-0.019	0.871–1.148	0.192
AMH	0.001	0.778–1.285	0.732
Hormone concentrations on HCG	0.000	0.487–2.052	0.000
E2(pmol/L)	0.088	0.559–1.788	0.010
P(nmol/L)	0.797	0.749–1.335	0.000
P(nmol/L)	-0.034	0.937–1.067	0.060
No. of ≥ 14mm folliculars	-0.019	0.871–1.148	0.192

Discussion

The findings of our study suggest that the level of LH_{HCG} can affect the IVF/ICSI outcome in the follicular-phase long protocol in a young population.

The “two cell–two gonadotropin” model has been the key to our understanding of folliculogenesis (4). According to this model, LH stimulates theca cells, thereby advancing androgen production, and FSH governs the proliferation of granulosa cells (GCs) and promotes E2 synthesis. Both FSH and LH play an important role in folliculogenesis. The details of the specific role of LH in folliculogenesis have not been fully studied.

The follicular-phase long protocol uses a single dose of 3.75 mg GnRH-a in the early follicular phase for pituitary downregulation. Two weeks after a single injection of 3.75 mg GnRH-a, endogenous sex hormones are almost completely inhibited. The FSH levels gradually recover after 3–4 weeks, and the E_2 levels begin to rise after 7–8 weeks, whereas the inhibitory effect on LH lasts up to 8 weeks after administration (13). After 30 days, as the FSH level gradually recovers, gonadotropin (Gn) is used to stimulate the development of multiple follicles in the ovary. In the follicular-phase long protocol, due to pituitary desensitization, endogenous LH levels are very low in the later stage of stimulation. In addition, approximately 50% of patients undergoing IVF/ICSI using GnRH-A are LH deficient. Therefore, adding recombinant LH (rLH) to the process of COH may be beneficial to the outcome (3). Therefore, when the serum LH concentration after pituitary downregulation is between 0.5–2.5 U/L, we can obtain a normal ovarian response (14). Too high or too low is not ideal.

LH, which is well known for its importance in the late follicular phase, supports theca cells in the ovary to provide androgens and hormonal precursors for estradiol production. LH not only promotes the growth of larger follicles but also increases FSH activity in granulosa cells by increasing androgen synthesis (15). During the development of a dominant follicle, its dependence on FSH gradually declines, whereas its dependence on LH gradually increases. FSH's partial role is replaced by LH in the late follicular phase. This facilitates the selection and maintenance of the dominant follicle.

Physiological levels of LH are clearly important for follicle development. If the LH level is abnormal, it will lead to abnormal follicular development. Therefore, it was speculated that there should be an LH threshold window. The Asia-Pacific consensus on the application of LH in assisted reproductive treatment published in 2011 concluded that the reasonable threshold window for LH ranges from 1.2–5 IU/L (16). However, some studies found that in the process of COH, when the LH level was lower than 1.2 IU/L on the HCG day, it did not affect the clinical outcome (17), whereas for the follicular-phase long protocol, LH was very rarely elevated due to suppression. In this study, the LH concentration was greater than 10 IU/L in only 10 cases, and there was only 1 case with LH > 20 IU/L, and the P concentrations on the HCG day were all normal.

Our study found that in the follicular-phase long protocol, with an LH ≤ 0.5 IU/L on the hCG day, patients had a higher number of oocytes retrieved, a better embryo rate, and more embryos, but there was no significant difference in the live birth rate compared with the other groups. So we speculate that when the LH ≤ 0.5 IU/L, although there may be pituitary hypersuppression leading to inadequate LH secretion in the late follicular phase, it does not affect the outcome. This is similar to the study of Brjercke et al. (18), that is, a lower LH can satisfy the development of follicles. As long as less than 1% of LH receptors are occupied, they can play a normal physiological role (18). However, when there are older women, a low response, a slow response, etc. in the COH cycle, adding LH may be beneficial. With a normal ovarian response, a low LH concentration does not affect the fertilization outcome (19).

However, as LH increases, the numbers of oocytes retrieved, embryos, and high-quality embryos and the number of 2PN decreased gradually, which may be because excessive LH levels may cause the ovaries to secrete more androgens, resulting in follicular atresia and then fewer oocytes retrieved. However, the pregnancy rate did not decrease, which may be because the increase in LH level only affected the number of oocytes retrieved but did not affect the quality of the oocytes, the embryo development potential or endometrial receptivity. For PGT patients, it may be more suitable to control the LH at a lower level on the HCG day to retrieve more oocytes.

The main limitations of this article are as follows: 1. The current measurement of LH levels may not accurately reflect the true level of internal LH; 2. Our study mainly enrolled young people, which is a specific group. This may have a certain influence on the outcome; 3. According to follicular development in the later stage, HMG or rLH may be added, which may cause the conclusion to be inaccurate. The cumulative pregnancy rate and cumulative live birth rate were not calculated. The data sample is still not large enough, and additional prospective large-sample studies are needed to confirm this idea.

In conclusion, in the follicular-phase long protocol, the LH level is maintained at a low level throughout COH. From the data of our study, we should pay more attention to LH_{HCG}. Elevated LH levels on the hCG day may lead to a reduction in oocyte retrieval. However, even when LH ≥ 5 IU/L, approximately 10 oocytes are retrieved, which, for clinical purposes, is sufficient. However, those who undergo PGT may benefit more when the LH level is controlled within a certain range. Therefore, in the follicular-phase long protocol among young women, monitoring of LH_{HCG} has clinical applications.

Declarations

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Author' contributions

WJZ, ZZL. contributed to the conception and design of the study. MML JHL were involved in acquisition of date collection and analyzed data. YCG drafted the manuscript. All authors revised the article and gave their final approval of the submitted version.

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Availability of data and materials

The data sets used and/or analyzed during the current study are available

from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was approved by the ethics committee of the Third Affiliated Hospital of Zhengzhou University （2021-105）

Consent for publication

Not applicable.

Conflict of interest

The authors have nothing to disclose

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Figures

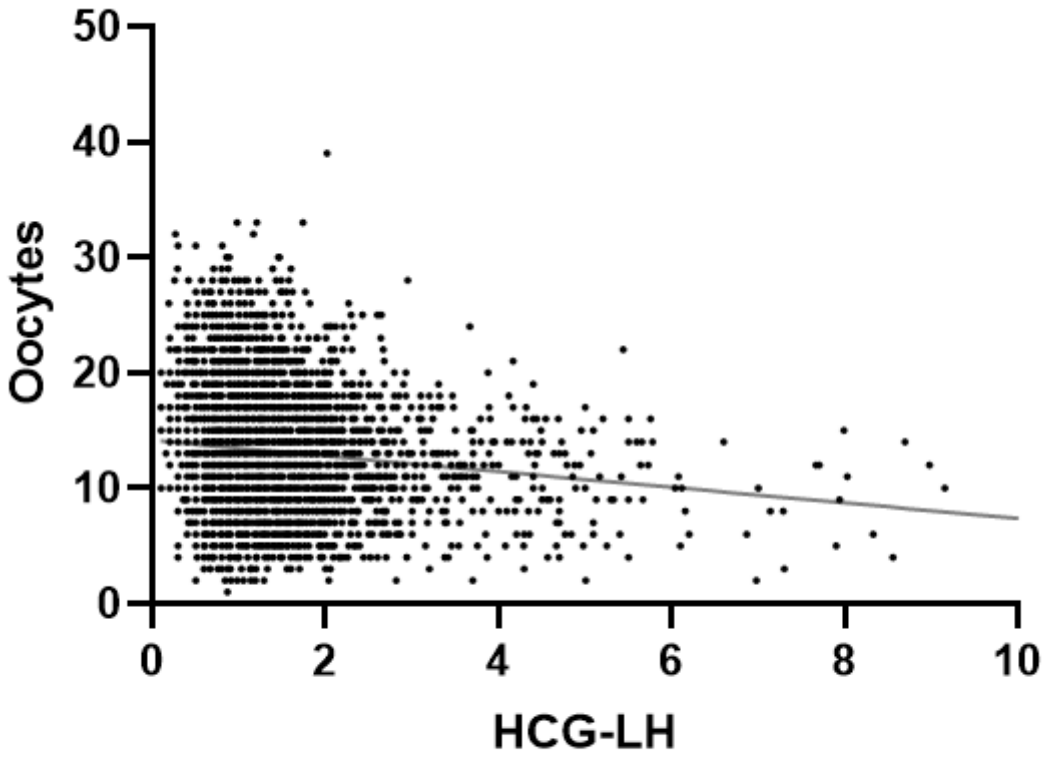

Figure 1

Linear relationship between serum LH concentration of HCG day and eggs retrieved The Figure showed that in the follicular-phase long protocol, with the increase of LHHCG, the number of retrieved eggs decreased.