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The impact of LH, E2, and P level of HCG administration day on outcomes of in vitro fertilization in controlled ovarian hyperstimulation

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Summary

Objectives: The objective of this study was to evaluate the impact of luteinizing hormone (LH), estradiol (E_2) and progesterone (P) levels on the day of human chorionic gonadotropin (HCG) administration on outcomes of in vitro fertilization (IVF) in controlled ovarian hyperstimulation (COH). *Study Design:* In this retrospective study, 129 infertile women undergoing IVF / intracytoplasmic sperm injection (ICSI) treatments were included; these cycles were stratified according to LH levels of ≥ 1.12 IU/L or < 1.12 U/L and according to E_2 levels of ≥ 1,005.89 pmol/L or < 1,005.89 pmol/L. The main outcome measure was the clinical pregnancy rate. *Results:* The clinical pregnancy rate was significantly higher in the group with LH ≥ 1.12 IU/L than in the group with LH < 1.12 U/L (43.28% vs. 30.65%, $p < 0.05$). The clinical pregnancy rate was also higher in the group with E_2 ≥ 1,005.89 pmol/L than in the group with average E_2 < 1,005.89 pmol/L (42.86% vs. 30.51%, $p < 0.05$). Among the LH, E_2, and P levels on the day of HCG administration, LH level was the most important predictor of outcomes of IVF in COH. The present data showed an adverse effect of low serum LH level (LH < 1.12 IU/L) on the day of HCG administration on clinical pregnancy rate. E_2 level can also predict the outcomes of IVF in COH. *Conclusions:* Low serum LH level (LH < 1.12 IU/L) and low serum E_2 level (average E_2 < 1,005.89 pmol/L) on the day of HCG administration led to low clinical pregnancy rates, while the P level on the day of HCG administration may have had little effect on clinical pregnancy.

Key words: In vitro fertilization-embryo transfer (IVF-ET); Controlled ovarian hyperstimulation (COH); Luteinizing hormone (LH); Estradiol (E_2); Progesterone (P).

Introduction

Improving the clinical pregnancy rate of in vitro fertilization/intracytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET) is currently the focus of many researchers.

Luteinizing hormone (LH) is the glycoprotein hormone secreted by the gonadotropin cells of the anterior pituitary. LH is known to be important in oocyte growth and maturation [1], it can promote the proliferation and differentiation of theca cells to produce androgen, which synergistically increases estrogen production. In the late follicular phase, LH helps produce small quantities of progesterone (P), thereby contributing to the promotion of positive estrogen feedback. The estradiol (E_2) level reflects follicle secretion and it is necessary for follicular development and maturation. Synthesis of E_2 is related to the development of dominant follicles, and serum E_2 concentration is the most meaningful indicator to assess follicular maturation. E_2 level relates to the number and size of follicles within the two sides of the ovaries. The average E_2 level of each follicle has greater predictive significance to determine the day of human chorionic gonadotropin (HCG) injection than the total E_2 level. It has been reported that the E_2 level could predict the outcome of IVF-ET treatment [2-4]. Serum P is mainly secreted by granular lutein cells and theca luteal cells. P can inhibit uterine contraction, reduce the sensitivity of the uterus to oxytocin, work against estrogen's function of endometrial proliferation to improve glandular secretion, and is conducive to embryo implantation and development. There are several reports regarding the impact of HCG administration day levels of LH, E_2, and P on the outcomes of IVF/ICSI-ET in controlled ovarian hyperstimulation (COH), but the results vary [2, 3, 5-11]. This study discussed the predictive value of serum LH, average E_2 level of follicles ≥ 14 mm in diameter and P level measured on the day of HCG administration on outcomes of IVF/ICSI-ET in COH, and determine the optimal time for HCG administration.

Materials and Methods

A total of 129 infertile women underwent IVF/ICSI-ET treatments at the Reproductive Medicine Center of NJPH from January 2010 to May 2011. Inclusion criteria were as follows: (1) younger than 40 years, (2) first cycle, (3) absence of moderate

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Table 1. — *Demographic information of groups of higher (A1) and lower (A2) LH levels on the day of HCG administration.*

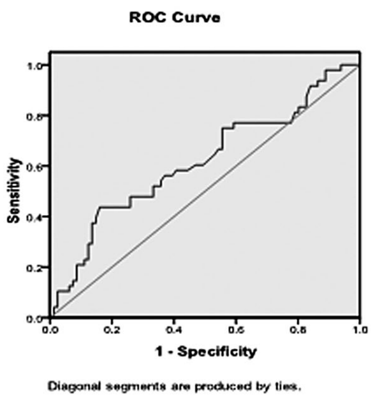
	Group A1 (LH ≥ 1.12 IU/L)	Group A2 (LH < 1.12 IU/L)	*p*
Number of patients	67	62	NS
Age, years	30.10 ± 4.40	29.66 ± 4.11	NS
Infertility period, years	4.34 ± 3.12	4.19 ± 2.68	NS
BMI	22.01 ± 2.53	22.16 ± 3.06	NS
Basal FSH, miu/ml	8.18 ± 2.53	7.87 ± 2.68	NS
Basal LH, miu/ml	4.54 ± 2.08	4.73 ± 2.84	NS
Basal E_2, pmol/L	185.61 ± 114.83	160.33 ± 81.28	NS
Basal PRL, ng/ml	17.06 ± 8.58	15.88 ± 8.44	NS
Basal T, ng/ml	1.79 ± 2.56	4.19 ± 19.35	NS
AFC	11.65 ± 3.81	12.77 ± 5.24	NS

Note: Continuous data are presented as the means ± SD.

Table 2. — *Demographic information of groups with higher (B1) and lower (B2) E_2 levels on the day of HCG administration.*

	Group B1 (average E_2 ≥ 1005.89 pmol/L)	Group B2 (average E_2 < 1005.89 pmol/L)	*p*
Number of patients	70	59	NS
Age, years	30.01 ± 4.73	29.75 ± 3.64	NS
Infertility period, years	4.30 ± 2.27	4.23 ± 2.43	NS
BMI	21.12 ± 2.36	21.24 ± 2.83	NS
Basal FSH, miu/ml	8.44 ± 2.57	7.54 ± 2.56	NS
Basal LH, miu/ml	5.03 ± 2.78	4.16 ± 1.95	NS
Basal E_2, pmol/L	184.77 ± 108.85	160.05 ± 88.75	NS
Basal PRL, ng/ml	16.72 ± 7.52	16.22 ± 9.59	NS
Basal T, ng/ml	3.96 ± 18.21	1.73 ± 2.75	NS
AFC	12.11 ± 3.52	12.40 ± 5.51	NS

Note: Continuous data are presented as the means ± SD.



Area Under the Curve

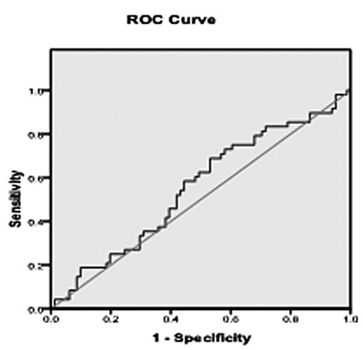
Test Result Variable(s): LH levels on the day of HCG

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.617	.052	.027	.514	.720

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Figure 1. — ROC curve of LH levels on the day of HCG administration.



Area Under the Curve

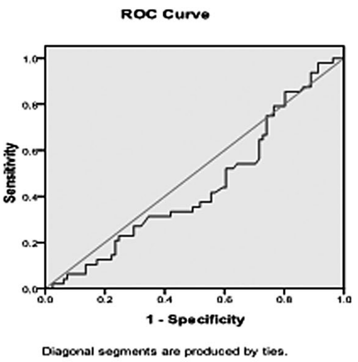
Test Result Variable(s): average E_2 on the day of HCG

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.552	.052	.327	.449	.654

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Figure 2. — ROC curve of average E_2 levels on the day of HCG administration.



Area Under the Curve

Test Result Variable(s): P levels on the day of HCG

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.443	.052	.280	.341	.545

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Figure 3. — ROC curve of P levels on the day of HCG administration.

or severe endometriosis, and severe hydrosalpinges, (4) absence of cancelled cases of implantation to prevent severe ovarian hyperstimulation syndrome (OHSS) or cases of OHSS. (5) Basal day 3 FSH level is under 10 miu/ml, and (6) the antral follicle count (AFC) > 6. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of NJPH.

Prior to treatment, all patients received 21 contraceptive pills followed by gonadotropin-releasing hormone agonist (GnRH-a) for down-regulation from day 17 to day 21 of the oral contraceptive regimen. FSH and/or human menopausal gonadotopin (HMG) were started on day 3 to day 6. HCG 10,000 IU was given on the night when four or more follicles reached ≥ 16 mm in diameter, three or more follicles reached ≥ 17 mm in diameter or two or more follicles reached ≥ 18 mm in diameter. Oocyte retrieval was performed 34-36 hours later, and ET was performed three days after oocyte retrieval. All patients received intramuscular P as luteal support from the date of oocyte retrieval. Blood HCG was measured 14 days after ET and Ultrasound was conducted 28 days after ET, and clinical pregnancy was defined as the presence of an intrauterine gestational sac and a fetal heart beat.

On the day of HCG administration, all patients had five ml of venous blood drawn at the Clinical Testing Center of NJPH between 7:45 and 8:00 am. The samples were centrifuged at 2,000 rpm for 15 minutes. Sera samples were tested using an immunoassay system automatic chemiluminescence analyzer and the immune serum chemiluminescence detection kit to determine the levels of serum LH, E_2, and P. These hormones on the HCG day were all within the normal range.

All data management and storage were completed with Reproductive Medicine Clinical Management System Software Version 9.9 of NJPH. SPSS 17.0 was used to conduct analyses, including ROC curve statistics, Student's t-test and x^2 test, and p values < 0.05 were considered to be statistically significant. Data are presented as the mean ± standard error of the mean.

Results

Of 129 fresh ET cycles, the causes of infertility included the following: tubal or pelvic factors (76 cycles, 58.91%), male factor (19 cycles, 14.73%), mild endometriosis (three cycles, 2.33%), unknown causes (six cycles, 4.65%), and combined male and female factors (25 cycles, 19.38%). Age, years of infertility, BMI, basic endocrine level, and AFC were not significantly different between groups (all $p > 0.05$; Tables 1 and 2).

In this study, there were 48 clinical pregnancies and 81 cases of pregnancy failure. According to the statistical ROC curves, the area under the curve for serum LH, average E_2 of follicles ≥ 14 mm in diameter and P levels on the day of HCG administration were 0.617, 0.552, and 0.443, respectively. Patients were divided into groups based on serum LH levels on the day of HCG administration as follows: one Group A1 with ≥ 1.12 IU/L and Group A2 with < 1.12 IU/L. The sensitivity of this measure to predict pregnancy was 0.60, and the specificity was 0.531. Similarly, patients were divided into groups based on the average E_2 levels of follicles ≥ 14 mm in diameter as follows: Group B1 with ≥ 1,005.89 pmol/L and Group B2 with < 1,005.89 pmol/L. The sensitivity of this measure to predict pregnancy was 0.625, and the specificity was 0.506. (ROC curves are shown in Figures 1, 2, and 3).

As shown in Table 3, groups with high and low LH levels show a statistically significant ($p < 0.05$) difference between the number of follicles ≥ 14 mm in diameter on the day of HCG administration and clinical pregnancy rates. Table 4 shows that groups with high and low E_2 levels for follicles ≥ 14 mm in diameter differ by LH and P levels, the number of follicles ≥ 14 mm and ≥ 16 mm in diameter on the day of HCG administration, and clinical pregnancy rate ($p < 0.05$). (Tables 3 and 4).

Table 3. — *Comparison of clinical outcomes between groups of higher (A1) and lower (A2) LH levels on the day of HCG administration.*

	Group A1 (LH ≥ 1.12 IU/L)	Group A2 (LH < 1.12 IU/L)	p
Number of patients	67	62	> 0.05
Start day of E_2, pmol/L	70.25 ± 53.89	70.99 ± 44.44	> 0.05
Start day of LH, IU/L	1.63 ± 0.77	1.37 ± 0.75	> 0.05
Start day of FSH, IU/L	3.28 ± 1.68	1.38 ± 0.75	> 0.05
Number of days using Gn	9.90 ± 1.57	10.32 ± 1.57	> 0.05
Gn usage, IU	2063.43±593.80	2196.37±701.84	> 0.05
E_2 levels on the day of HCG administration, pmol/L	9047.13 ± 4247.74	8285.50±4842.39	> 0.05
P levels on the day of HCG administration, nmol/L	4.02 ± 1.75	4.24±2.05	> 0.05
Average E_2 levels of follicles ≥ 14 mm on the day of HCG administration, pmol/ L	1480.47±958.45	1161.12±1405.40	> 0.05
Number of follicles ≥ 14 mm in diameter on the day of HCG administration	7.16 ± 3.68	8.85 ± 4.99	< 0.05
Number of follicles ≥ 16 mm in diameter on the day of HCG administration	3.75 ± 2.94	4.58 ± 3.59	> 0.05
Average number of aspirated follicles	15.12 ± 7.02	16.76 ± 9.35	> 0.05
Number of retrieved oocytes	11.45 ± 5.88	13.77 ± 7.49	> 0.05
Metaphase II fertilization rate, %	67.74 ± 16.36	69.72 ± 13.34	> 0.05
Metaphase II cleavage rate, %	95.94 ± 4.02	93.66 ± 3.17	> 0.05
Good quality embryo rate on day 3, %	62.76 ± 2.69	63.84 ± 3.84	> 0.05
Endometrial thickness on the day of embryo transfer, mm	10.76 ± 2.26	11.52 ± 2.45	> 0.05
Number of implanted embryos	1.88 ± 0.59	1.82 ± 0.56	> 0.05
Clinical pregnancy rate, %	43.28	30.65	< 0.05

Note: Continuous data are presented as the means ± SD; categorical data are presented as n (%).

Table 4. — *Comparison of clinical outcomes between higher (B1) and lower (B2) E_2 levels of follicles ≥14 mm in diameter on the day of HCG administration.*

	Group B1 (average E_2 ≥ 1,005.89 pmol/L)	Group B2 (average E_2 < 1,005.89 pmol/L)	p
Number of patients	70	59	> 0.05
Start day of E_2, pmol/L	70.15 ± 44.45	71.14 ± 55.06	> 0.05
Start day of LH, IU/L	1.49 ± 0.71	1.53 ± 0.83	> 0.05
Start day of FSH, IU/L	2.59 ± 1.41	2.49 ± 0.99	> 0.05
Number of days using Gn	10.09 ± 1.56	10.12 ± 1.61	> 0.05
Gn usage, IU	2118.93±595.29	2137.29±712.28	> 0.05
P levels on the day of HCG administration, pmol/L	4.51 ± 1.92	3.67 ± 1.78	< 0.05
LH levels on the day of HCG administration, nmol/L	1.84 ± 1.42	1.21 ± 0.82	< 0.01
Number of follicles ≥ 14 mm in diameter on the day of HCG administration	6.63 ± 3.43	9.58 ± 4.94	< 0.01
Number of follicles ≥ 16 mm in diameter on the day of HCG administration	3.30 ± 2.46	5.15 ± 3.82	< 0.01
Number of aspirated follicles	15.48 ± 8.20	16.38 ± 8.20	> 0.05
Number of retrieved oocytes	12.63 ± 7.02	12.49 ± 6.55	> 0.05
Metaphase II fertilization rate, %	67.85 ± 16.69	69.68 ± 13.36	> 0.05
Metaphase II cleavage rate, %	94.40 ± 5.52	95.38 ± 4.47	> 0.05
Good quality embryo rate on day 3, %	63.56 ± 3.59	62.95 ± 2.97	> 0.05
Endometrial thickness on the day of embryo transfer, mm	10.84 ± 2.36	11.46 ± 2.37	> 0.05
Number of implanted embryos	1.84 ±0. 61	1.86 ± 0.54	> 0.05
Clinical pregnancy rate, %	42.86	30.51	< 0.05

Note: Continuous data are presented as the means ± SD; categorical data are presented as n (%).

Discussion

Only when serum LH levels are above the threshold of LH in the body can LH maintain normal follicle development and hormone secretion [12]. Some researchers have proposed that appropriately complementary LH helps to improve the pregnancy outcome of IVF in some patients [13], while other researchers have argued that normal women with significantly lower LH levels after down-regulation of COH can still obtain a good pregnancy outcome [14].

Fleming *et al.* found that when LH < 1 IU / L in the mid-follicular phase, E_2 levels decreased, thus leading to adverse pregnancy outcomes [14]. Lu *at al.*'s study showed that the clinical pregnancy rate (58.33%) when serum LH was ≥ 1.58 IU / L on the day of HCG administration was significantly higher than when LH was <1.58 IU / L (34.48%) [15]. A recently meta-analysis showed that the addition of recombinant LH to IVF cycles may improve implantation and clinical pregnancy in patients of advanced reproductive age [16].

In the present study, the authors found that group A2 had more follicles ≥ 14 mm and ≥ 16 mm in diameter than group A1 ($p < 0.05$), the clinical pregnancy rate was also lower than group A1 ($p < 0.05$). These results suggest that there is an specifically response in group A1 in the process of ovulation as a moderate amount of LH can prevent estrogen decline and reduce FSH dosage. A lack of LH may affect follicular development, resulting in the specifically poor response to FSH. Fleming's study suggested that low serum levels of LH were not conducive to follicular growth but could reduce the differences in follicular development; the FSH dose increases, while the number of retrieved oocytes increases with increased administration time of FSH [17]. A moderate LH level is important for the maturation of oocytes and maintenance of luteal functions. This research offered some basis for the evaluation of moderate serum LH level of <1.12 IU / L on the day of HCG administration in COH and found that the clinical pregnancy rate in this group decreased significantly compared to the other groups studied.

In COH, injecting HCG when the average E_2 level of each mature follicle reached 732-1,098 pmol/L achieved a maximum rate of embryo implantation and clinical pregnancy, while the lowest clinical pregnancy rate was reported with E_2 levels below 732 pmol/L [2]. It has been reported that E_2 > 2,936 pmol/L leads to a higher pregnancy rate, but an overly high level of E_2 is not conducive to embryo implantation. Others have suggested that the E_2 level on the day of HCG administration or E_2 pretreatment has no correlation with pregnancy rate [6, 18-21]. While another paper showed that serum E_2 levels had a concentration-dependent effect on the pregnancy outcome, suggesting an optimal range of E_2 level for achieving a successful pregnancy: 3,000-4,000 pg/ml for women < 38 years and 2,000-3,000 pg/ml for women ≥ 38 years [22].

In the present study, the authors found that group B1 had higher LH and P levels on the day of HCG administration day compared to group B2 ($p < 0.05$). Between groups B1 and B2, the difference in the number of follicles ≥ 14 mm or ≥ 16 mm in diameter on the day of HCG administration was statistically significant ($p < 0.01$). As the number of follicles significantly increased in group B2, it may be inferred that an excessive number of follicles resulted in a decline in the average E_2 level of each follicle, which may affect the quality of follicles. Therefore, individualized treatments are needed in the process of COH to avoid the growth of too many follicles, which not only increases the risk of ovarian hyperstimulation but also has an adverse impact on pregnancy outcome. There was a significant difference in the number of follicles ≥ 14 mm on the day of HCG administration between groups B1 and B2 ($p < 0.05$). The significant reduction in the number of follicles in the group with a higher clinical pregnancy rate suggests a relationship between the number of follicles and clinical pregnancy. The clinical pregnancy rate was lower in group B2 than in group B1 ($p < 0.05$). Synchronizing the process of IVF/ICSI-ET is the key to improving the clinical pregnancy rate. A low E_2 level may not adequately increase the number of spiral vessels, glands, and glandular secretion after ovulation, thus reducing endometrial perfusion with inadequate activation of cytokines and causing follicular and endometrial development to be asynchronous. The present authors found that in COH, the average E_2 level of follicles ≥14 mm in diameter on the day of HCG administration was related to pregnancy outcome and that the clinical pregnancy rate was significantly lower in the group with an average E_2 level < 1,005.89 pmol/L.

Endometrial receptivity is an important factor affecting IVF-ET pregnancy outcomes. Studies suggest that serum P level on the day of HCG administration in IVF-ET did not affect the follicular quality but affected endometrial receptivity, resulting in lower clinical pregnancy rates and implantation rates [10, 21].

According to the ROC curves generated in the present study, the area under the curve of serum P level on the day of HCG administration was 0.443. This suggests that the serum P level on the day of HCG administration may have no effect on pregnancy outcomes of IVF/ICSI-ET. However, according to Ochsenkühn *et al.*, live birth rate in cycles with GnRH-a was significantly lower in women with P levels ≥ 2.0 ng/ml (17.4%) on the day of HCG administration as compared with women with P levels < 1.5 ng/ml (24.6%) and 1.5-1.99 ng/ml (26.7%) [23]. One new study reported that P/E2 ratio on day of HCG administration improves the prediction of IVF outcome when compared to serum P levels alone [24]. So further studies are needed to elucidate this issue.

In summary, this retrospective study arrived at the preliminary conclusion that among the three serum markers, LH level, average E_2 level of follicles ≥14 mm in diameter, and P level on the day of HCG administration, serum LH level had the strongest predictive value for pregnancy outcome. When serum LH level was < 1.12 IU/L on the day of HCG administration, the clinical pregnancy rate following IVF/ICSI-ET was significantly lower. At the same time, using ROC curves, the present authors concluded that when serum LH levels were greater than 2.14 IU/L, 87.7% of IVF/ICSI-ET patients had negative pregnancy outcomes. Therefore, serum LH level should be maintained within a certain range during the process of COH; when LH < 1.12 IU/L, adding supplemental LH may help to further increase the pregnancy rate. Monitoring the LH level may also help to determine the optimal time to inject HCG, with the goal to further enhance the pregnancy rate among patients undergoing IVF/ICSI-ET. In COH cycles, the average E_2 level of follicles ≥ 14 mm in diameter on the day of HCG administration was related to pregnancy outcome; when average E_2 level < 1,005.89 pmol/L, clinical pregnancy rate was significantly lower. This study indicated that serum P level on day of HCG administration may have had little effect on the pregnancy outcomes of IVF/ICSI-ET.

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