The Association Between LH Serum Levels at The Time of hCG Injection and Clinical Pregnancy during IVF

Budi Wiweko, Bram Pradipta, Muharam Natadisastra, Kanadi Sumapraja, Eliza Mansyur, Tita Yuningsih, Andon Hestiantoro

Department of Obstetrics and Gynecology Faculty of Medicine Universitas Indonesia Dr. Cipto Mangunkusumo General Hospital

Abstract

Some studies showed that recombinant follicle stimulating hormone (r-FSH) administration without LH supplementation has a good success rate on in vitro fertilization (IVF). However, LH role in IVF has been controversial. This study was conducted to evaluate the association between serum LH levels at the time of hCG injection and clinical pregnancy during IVF. This retrospective study included 136 patients underwent long protocol IVF from January 2005 to December 2009 in Yasmin Clinic, Cipto Mangunkusumo Hospital, Jakarta. The controlled ovarian hyper-stimulation protocol used in this study was gonadotropin-releasing hormone (GnRH) agonist and r-FSH. Serum LH level was measured on the day of hCG administration and the outcome evaluated was the clinical pregnancy following IVF cycles. There were 32 IVF cycles resulted in pregnancy out of 136 cycles (23.5%). There was significant difference between serum LH levels on the day of hCG administration and clinical pregnancy outcome (p=0.036). LH serum level cut-off value of ≥1.050 IU/l was the best value to predict pregnancy outcome with 62.5% sensitivity, 59.4% specificity, 83.3% positive predictive value, and 32.76% negative predictive value. It was concluded that there was an association between serum LH levels at the time of hCG injection and clinical pregnancy during IVF.

Keywords: *IVF cycles, LH levels serum, hCG administration*

Hubungan Kadar LH saat Penyuntikan hCG dengan Kejadian Kehamilan pada Fertilisasi In vitro

Abstrak

Pemberian follicles stimulating hormone rekombinan (r-FSH) tanpa suplementasi LH memiliki tingkat keberhasilan fertilisasi in vitro (FIV) yang baik namun, peran LH pada FIV masih kontroversial. Tujuan penelitian ini adalah untuk mengevaluasi hubungan antara kadar serum LH pada hari penyuntikan hCG dengan kehamilan klinis selama FIV. Penelitian retrospektif ini melibatkan 136 perempuan yang menjalani FIV siklus pertama dengan protokol panjang mulai bulan Januari 2005 sampai Desember 2009 di klinik Yasmin, Rumah Sakit dr. Cipto Mangunkusumo, Jakarta. Protokol hiperstimulasi ovarium yang digunakan adalah agonis gonadotropin-releasing hormone (GnRH) dan r-FSH. Kadar LH serum diukur pada hari pemberian hCG dan outcome penelitian yang dievaluasi adalah kehamilan klinis. Kehamilan terjadi pada 32 siklus FIV dari 136 siklus (23,5%). Terdapat perbedaan bermakna antara kadar serum LH pada hari penyuntikan hCG dengan kehamilan klinis (p = 0,036). Titik potong kadar serum LH ≥1,050IU/l adalah nilai terbaik untuk memprediksi hasil kehamilan dengan sensitivitas 62,5%, 59,4% spesifisitas, nilai prediksi 83,3% positif, dan nilai prediksi 32,76% negatif. Disimpulkan bahwa terdapat hubungan antara kadar serum LH pada hari penyuntikan hCG dengan kehamilan klinis selama FIV.

Kata kunci: *siklus IVF, kadar LH serum, pemberian hCG*

Introduction

LH is a glycoprotein hormone that plays an important role in the process of steroidogenesis. The concept of *two cell – two gonadotropin* explains that granulosa and theca cell stimulation by LH and FSH results in intra- follicular estradiol production that affects follicle maturation.[1] Optimal level of LH is necessary for ovum maturation, fertilization process, and embryonic development. If LH level is too low, it will inhibit follicle maturation and if it is too high (e.g premature LH surge), it will increase the risk of miscarriage. [2-4]

IVF is an infertility treatment that has the highest clinical pregnancy rate, which is around 20-50%. In women with fallopian tube abnormalities (e.g. obstruction and infection), IVF is the best option. Two main factors determining the success of IVF are the quality of embryos and endometrial receptivity. The quality of embryos are determined by various factors including the reproductive hormones level (e.g. FSH, estradiol, LH, prolactin, progesterone).[5]

One of the developments in IVF methods is the use of r-FSH. Studies results showed that the use of r-FSH alone without LH supplementation has good IVF success rate. Thus, LH role in IVF is still controversial. The optimal LH level for IVF success has not been determined.[3,6] This study aims to investigate the relationship between LH levels during hCG injection with IVF outcome. The results are expected to optimize the IVF success rate.

Methods

This retrospective study was conducted on 136 infertility patients who underwent IVF cycles in Yasmin Clinic, dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia from January 2005 to December 2009. The inclusion criterion was infertile patients who completed the long protocol IVF cycles. Patients with history of previous ovarian surgery were excluded.

Controlled ovarian hyper-stimulation protocol used in this study was the long protocol. GnRH agonist 0.5 mg was administered during mid-luteal phase for 14 days. Afterwards, the dose was reduced to 0.25 mg and 225 IU r-FSH was added for 10-12 days. Recombinant human chorionic gonadotropin (hCG) 250 mg was administered if ultrasonography (USG) examination showed follicular diameter ≥20 mm or endometrial thickness ≥10 mm. The hormone hCG was given one or two days before ovum pick up (OPU). On the day of hCG injection LH serum level was measured by ELISA using Immulite machine from Siemens.

Clinical pregnancy was defined as the presence of the gestational sac inside the uterus in three to four weeks after OPU. The data was analyzed using SPSS software 16.00. Student's t-test was used to compare LH serum levels on the day of hCG injection with IVF outcome (i.e. clinical pregnancy) for data with normal distribution. Mann-Whitney U-test was used to analyze data without normal distribution. P-value of <0.05 is considered statistically significant. Area under the receiver operating characteristic curve (AUC) and odds ratio (OR) were calculated to determine the power of the analyzed variables.

Results

There were 136 subjects with age varied between 22-48 years old who underwent IVF cycle and fulfilled the inclusion criteria (Table 1). There were 136 IVF cycles resulting in 32 pregnancies (23.5%). Most subjects were experiencing primary infertility (97.1%).

Based on Kolmogorov Smirnov normality test, it was shown that the data did not have normal distribution therefore Mann-Whitney U-test was used to determine the relationship between LH levels on the day of hCG injection and the occurrence of pregnancy. We found a significant correlation between LH levels on the day of hCG injection and the occurrence of pregnancy (p =0.036)

Based on analysis receiver operating characteristic (ROC), the threshold value of LH levels on the day of hCG injection to determine the clinical pregnancy was ≥1.050 IU/l. LH levels ≥1.050 IU/l has a sensitivity of 62.5%, specificity of 59.4%, positive predictive value of 83.3%, and 32.76% negative predictive value in determining the clinical pregnancy. LH levels ≥1.050 IU/l on the day of injection of hCG association with pregnancy rate was almost 0.4 times lower than that of LH levels <1.050 IU/l (OR 0.411; AUC 0.623; Figure 1).

Table 1. Demographic Data of Pregnant and Not Pregnant IVF Patients

Variable	Pregnant (n=32)	Not pregnant (n=104)	p
Age (year)	33,31	34,90	0.065
Infertility type			
Primary infertility	27 (84.4%)	101 (97.1%)	0.007
Secondary infertility	5 (15.6%)	3 (2.9%)	
Duration of infertility (year)	7.22	7.05	0.825
Causes of infertility (female factor)			
Tubal factor	8 (25%)	21 (20.2%)	0.292
Endometriosis	0 (0%)	14 (13.5%)	
Ovulation disfunction	3 (9.4%)	7 (6.7%)	
Other factors	4 (12.5%)	12 (11.5%)	
Idiopatic	17 (53.1%)	50 (48.1%)	
Causes of infertility(male factor)			0.108
Azoospermia	1 (3.1%)	18 (17.3%)	
Severe Oligospermia	13 (40.6%)	31 (29.8%)	
Other factors	18 (56.3%)	55 (52.9%)	
Insemination method			
ICSI	31 (96.9%)	85 (81.7%)	0.106
Conventional	0 (0%)	1 (1%)	
Combination (ICSI+conventional)	1 (3.1%)	18 (17.3%)	
FSH basal hormone level (IU/l)	6.33	7.19	0.211
AMH basal hormone level (µg/ml)	4.43	4.64	0.755
Progesteron level on the day of hCG injection (ng/ml)	1.41	1.61	0.755
Estradiol level on the day of hCG injection (pg/ml)	2.47	2.14	0.137
LH level on the day of hCG injection	1.07	1.57	0.02
Endometrial thickness on the day of hCG injection	11.61	10.76	0.048
Total mature follicular (≥17mm) on OPU	8.13	7.38	0.306
Total mature oocyte (2nd metaphase oocyte)	7.81	6.81	0.167

A total of 58 subjects (42.6%) had LH levels <1.050 IU/l after stimulation. A total of 59.4% subjects with LH levels <1.050 IU/l showed clinical pregnancy compared to 40.6% subjects with LH levels ≥1.050 IU/l (Table 2). There was significant difference in term of clinical pregnancy between patients with LH level ≥1.050 IU/l and those with LH level <1.050 IU/l (p = 0.029)

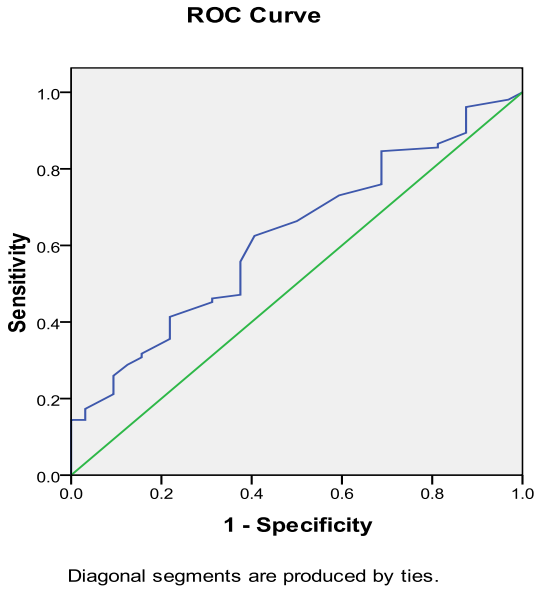

Figure 1. Sensitivity and SLH Level on hCG Injection with IVF Outcomes

Table 2. Pregnancy Outcomes According to LH Level on the Day of hCG Injection

LH (IU/l)	Pregnant (%)	Not pregnant (%)
<1,050	19 cycles (59.4)	39 cycles (37.5)
≥1,050	13 cycles (40.6)	65 cycles (62.5)
Total	32 cycles (100.0)	104 cycles (100.0)

Discussion

Gonadotropic hormones, LH and FSH, work synergistically in the process of follicular development and maturation.[3] Nevertheless, the role of LH in follicle stimulation during follicular phase in patients undergoing IVF cycle is still being debated. According to Chappel et al[7] study LH had little role in the process of follicle maturation during the follicular phase. Moreover, the optimal level of LH that supports IVF success have not yet been determined.[3]

This study determined LH levels threshold value of ≥1.050 IU/l on the presence of clinical pregnancy based on ROC analysis. LH level ≥1.050 IU/l had a 62.5% sensitivity, 59.4% specificity, 83.3% positive predictive value, and 32.76% negative predictive value in determining the pregnancy outcomes. This threshold value is equivalent to the threshold values in researches conducted by Westergaard et al[8] which was less than 0.5 IU/l and by Balasch et al[9] who determined the threshold value of LH levels less than 1, 0.7, and 0.5 IU/l. The comparison between sensitivity, specificity, negative predictive value, and positive predictive value were not observed in those previous researches.

A total of 58 subjects (42.6%) experienced a decrease in LH concentration to <1.050 IU/l on the day of hCG injection. To the best our knowledge, there is not any other research that measured the levels of LH on the day of hCG injection. Westergaard et al.[8] conducted a study with 200 normo-gonatodotropic subjects who underwent IVF with GnRH agonist and r-FSH. In their study 49% of subjects had of LH levels of <0.5 IU/l on mid-follicular phase. Another study was carried out by Balasch et al[9] with 144 subjects who underwent IVF with 0.1 mg GnRH agonist (i.e. triptorelin acetate) and 300-450 IU r-FSH. As many as 31%, 15%, and 17% subjects had higher LH levels of <1, ≤0.7, and <0.5IU/l respectively at mid-follicular phase.

A total of 59.4% subjects with LH levels <1.050 IU/l showed clinical pregnancy compared to 40.6% subjects with LH levels ≥ 1.050 IU/l. The association with pregnancy rate when LH level of ≥ 1.050 IU/l on the day of hCG injection is almost 0.4 times lower than when LH levels <1.050 IU/l (OR 0.411; AUC 0.623; (Figure 1)).

Previous researches regarding LH level used different threshold values with varied results. Some researches suggested that low level of LH was associated with lower incidence of pregnancy. The study by Westergaard et al[8] stated that the incidence of pregnancy in subjects with low LH levels were 30% compared to 34% in subjects with normal LH levels (> 0.5 IU/l). Another study by Balasch et al[9] showed that 20% of subjects with LH levels <1 IU/l experienced a clinical pregnancy compared to 52% of subjects with LH levels> 1 IU/l. Esposito et al[1] conducted a study to 166 normo-gonadotrophic subjects who underwent IVF cycles with leuprolide acetate and r-FSH. The study showed that clinical pregnancy occurred in 40% of subjects with LH levels < 3mIU/mL and in 43% subjects with LH levels ≥ 3 mIU/mL.

A different result was reported by Humaidan et al[10] who conducted a study on 207 patients using LH threshold value of ≤ 0.5 IU/l LH, from 0.51 to 1.0 IU/l, from 1.01 to 1.5 IU/l, and>1.5 IU/l. In their study, the most optimal outcome was observed in group with LH threshold value between 0.51 to 1.5 IU/l. Patients whose LH levels were higher than that had lower incidence of pregnancy.

In this research we found a significant correlation between LH level and pregnancy outcome (p = 0.036). In addition, there was significant different in term of clinical pregnancy between subjects with LH levels ≥ 1.050 IU/l and subjects with LH levels <1.050 IU/l (p = 0.029). Similar studies by Esposito et al,[1] Westergaard et al,[8] and Balasch et al[9] found no relationship between LH levels and pregnancy as the outcome of IVF.

Differences between the results of this study compared to the previous researches were due to several reasons. Firstly, there are differences in the dose of GnRH agonist and r-FSH in the controlled ovarian hyperstimulation protocol used in each study. Secondly, the different GnRH regiment used is considered to be responsible for the various effect of GnRH on ovarian steroidogenesis suppression. Thirdly, time of measurement and the threshold value of LH levels applied were varied in previous researches.[3,8,9]

Conclusion

LH concentration threshold value of ≥ 1.050 IU/l has 62.5% sensitivity, 59.4% specificity, 83.3% positive predictive value, and 32.76% negative predictive value in determining pregnancy outcomes. There was significant correlation between LH levels on the day of hCG injection and the occurrence of pregnancy (p=0.036). In addition, there was significant difference in pregnancy incidence between subjects with LH levels ≥ 1.050 IU/l and subjects with LH levels <1.050 IU/l (p = 0.029). LH concentration on the day of hCG injection can be useful in predicting incidence of pregnancy as the outcome of IVF.

References

1. Esposito MA, Barnhart KT, Coutifaris C, Patrizio P. Role of periovulatory luteinizing hormone concentrations during assisted reproductive technology cycles stimulated exclusively with recombinant follicle-stimulating hormone. Fertil Steril. 2001;75(3):519-24.
2. American Society for Reproductive Medicine. Effect of midfollicular luteinizing hormone levels on ovarian response and pregnancy outcome in patients undergoing in vitro fertilization in a short-term protocol. Fertil Steril. 2005;83:1043-6.
3. Penarubia J, Fabregues F, Creus M, Manau D, Casamitjana R, et al. LH serum levels during ovarian stimulation as predictors of ovarian response and assisted reproduction outcome in down-regulated women stimulated with recombinant FSH. Hum Reprod. 2003;18(12):2689-97.
4. Gordon UD, Harrison RF, Fawzy M, Hennelly B, Gordon AC. A randomized prospective assessor-blind evaluation of luteinizing hormone dosage and in vitro fertilization outcome. Fertil Steril. 2001;75(2):324-31.
5. Strowitzki T, Germeyer A, Popovici R, Wolff M. The human endometrium as a fertility-determining factor. Hum Reprod. 2006;12:617-30.
6. Chappel SC, Howles C. Re-evaluation of the roles of luteinizing hormone and follicle-stimulating hormone in the ovulatory process. Hum Reprod. 1991;6:1206-12.
7. Westergaard LG, Laursen SB, Andersen CY. Increased risk of early pregnancy loss by profound suppression of luteinizing hormone during ovarian stimulation in normogonadotrophic women undergoing assisted reproduction. Hum Reprod. 2000;15:1003-8.
8. Balasch J, Vidal E, Penarrubia J, Casamitjana R, Carmona F, et al. Suppression of LH during ovarian stimulation: analyzing threshold values and effects on ovarian response and the outcome of assisted reproduction in down-regulated women stimulated with recombinant FSH. Hum Reprod. 2001;16(8):1636-43.
9. Humaidan P, Bungum L, Bungum M, Yding AC. Ovarian response and pregnancy outcome related to mid-follicular LH levels in women undergoing assisted reproduction with GnRH agonist down-regulation and recombinant FSH stimulation. Hum Reprod. 2002;17:2016-21.
10. Kolibianakis EM, Zikopoulos K, Schiettecatte J, Smitz J, Tournaye H, et al. Profound LH suppression after GnRH antagonis administration is associated with a significantly higher ongoing pregnancy rate in IVF. Hum Reprod. 2004;19(11):2490-6.