Original Article

Impact of Serum Human Chorionic Gonadotropin and Luteinizing Hormone Receptor Expression to Oocyte Maturation Rate: A Study of Controlled Ovarian Stimulation

Hilwah Nora[1,2], Budi Wiweko[2,3,4], R. Muharam[2,3,4], Rajuddin[1], Grace Wangge[5], Andon Hestiantoro[2,3,4], Gita Pratama[2,3,4], Achmad Kemal Harzif[2,3,4], Sarah Chairani Zakirah[2,3]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, University of Syiah Kuala, Banda Aceh, [2]Department of Obstetrics and Gynecology, Faculty of Medicine, University of Indonesia, [3]Human Reproductive Infertility and Family Planning, Indonesian Medical Education and Research Institute, [4]Yasmin IVF Clinic, Dr. Cipto Mangunkusumo General Hospital, [5]Southeast Asia Ministers of Education Regional Centre for Food and Nutrition – Pusat Kajian Gizi Regional, University of Indonesia, Central Jakarta, Indonesia

ABSTRACT

Background: Few studies have assessed the impact of serum human chorionic gonadotropin (hCG) levels before oocyte retrieval and luteinizing hormone receptor (LHR) mRNA expression. **Aims:** The objective was to assess the correlations between serum hCG levels at 12-h posttrigger granulosa cell LHR mRNA expression during the *in vitro* fertilization (IVF) cycle with oocyte maturation rate and to determine the cutoff level of serum hCG at 12-h posttrigger. **Settings and Design:** A cross-sectional was conducted for this study at the IVF center of Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia. **Materials and Methods:** Recombinant follicle-stimulating hormone was used on day 2 of the menstrual cycle with multiple doses of a gonadotropin-releasing hormone antagonist. Recombinant hCG was used to trigger ovulation. At 12-h posttrigger, hCG serum levels were measured using an enzyme-linked immunosorbent assay. **Statistical Analysis:** Pearson's correlation coefficient was used to evaluate the correlation between oocyte maturation rates, serum hCG levels, and LHR mRNA levels. Cutoff values were determined using a receiver operating characteristic (ROC) curve. **Results:** Serum hCG levels were positively correlated ($r = 0.467$; $P < 0.01$), and LHR mRNA expression was weakly correlated ($r = 0.073$; $P = 0.701$) with oocyte maturation. The cutoff of serum hCG for a high maturation rate was 77 mIU/mL, with an area under the ROC curve of 0.765 (95% confidence interval: 0.598–0.939) and $P < 0.001$. **Conclusion:** Oocyte maturation is correlated with serum hCG levels with 77 mIU/mL as the cutoff point for oocyte retrieval.

KEYWORDS: *Cutoff value, human chorionic gonadotropin, luteinizing hormone receptor, oocyte maturation, posttrigger*

BACKGROUND

Although many studies have assessed human chorionic gonadotropin (hCG) levels after hCG administration, the cutoff value at 12-h posttrigger regarding the reproductive outcome is inconsistent. Therefore, the hCG level posttrigger might be correlated with the reproductive outcome.[1] The aim of the present study was to investigate the correlation between serum hCG levels at 12 h after recombinant hCG (rhCG) trigger and granulose cell luteinizing hormone receptor (LHR) mRNA expression with oocyte maturation rate and to determine the optimal cutoff level of serum hCG at 12-h posttrigger.

MATERIALS AND METHODS

Subjects selection

The study cohort included 30 women who underwent Assisted Reproductive Technology (ART) cycles at

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Address for correspondence: Prof. Budi Wiweko,
Indonesian Medical Education and Research Institute,
Salemba Raya No. 6 Street, Central Jakarta 10430, Indonesia.
E-mail: wiwekobudi@yahoo.co.id

Dr. Cipto Mangunkusumo General Hospital, Jakarta who met the following inclusion criteria: signed an informed consent to be included in this study, which had been informed about the process of ovarian stimulation, oocyte extraction, side effects, and sample processing; a normoresponder which defined by normal ovarian reserve test (anti-Mullerian hormone 1.2–4.5 ng/ml or antral follicle count 6–19 follicles/ovary), used a gonadotropin-releasing hormone antagonist protocol of controlled ovarian stimulation by recombinant follicle-stimulating hormone (rFSH) 225 IU, and triggered with rhCG to obtain oocyte maturation. The exclusion criteria were the presence of comorbidities, such as endometriosis and polycystic ovary syndrome and smoking history.

Patient characteristics

Patient age, cause of infertility, body mass index (BMI), anti-Müllerian hormone level, and the total dose of FSH were recorded. Data were obtained from the patient medical records. Causes of infertility based on data from medical records and diagnostic procedures for new patients were categorized as tubal factors, male factors, or unexplained. The total number of oocytes, immature oocytes, and mature oocytes, morphology score, and maturation rate were evaluated by an embryologist using a microscope. The maturation rate was quantified by dividing mature oocyte with a total oocyte. Low maturation rate obtained if the <75% score was achieved after rhCG injection. While normal maturation rate obtained if the ≥75% score was achieved. LHR expression of granulosa cells was quantified using quantitative reverse transcription polymerase chain reaction (qRT-PCR). At 12-h posttrigger, hCG serum levels were measured using an enzyme-linked immunosorbent assay (ELISA).

Oocyte extraction

The procedures of *in vitro* fertilization were ovarian stimulation, oocyte retrieval, embryo transfer, and luteal phase support. However, we only did ovarian stimulation with rFSH and rhCG, then oocyte retrieval to observe oocyte maturation rate, LHR mRNA expression and to determine cutoff serum hCG level at 12 h after triggering with rhCG. Each oocyte was stimulated with rFSH (Gonal F; Merck Serono, Geneva, Switzerland) on day 2 of the menstrual cycle with multiple doses of a gonadotropin-releasing hormone antagonist. rFSH (Cetrotide; Merck Serono) was used to suppress pituitary hormone production. Monitoring of follicular development was performed using transvaginal ultrasound and measurement of serum estradiol levels. rhCG (Ovidrel; Serono), at a dosage as high as 250 μg, was used to trigger ovulation when the size of three or

more follicles was ≥18 mm. The morning following hCG administration, serum hCG levels were measured using an ELISA. The time interval between hCG injection and blood sampling for the measurement of serum hCG levels was 12 h. Ultrasound guidance was used to retrieve oocytes at 35–36 h after hCG injection. The benchmark of oocyte retrieval is the presence or absence of germinal vesicles (GVs) or the first polar body, as assessed with a stereomicroscope at ×200 magnification. In some cases, as much as 85 IU/mL of hyaluronidase was used to enhance visualization of oocyte maturity.

Oocytes captured at either prophase I (oocytes with GVs) or metaphase I (evidence of GV breakdown, but no visible polar body) are considered to be immature. mRNA was pooled from granulosa cells from both ovaries. Transcriptome analysis of total RNA was performed by microarray hybridization. Granulosa cells were collected during oocyte retrieval for intracytoplasmic sperm injection procedures, and pure RNA was extracted and analyzed by qRT-PCR.

Study approval

The study protocol was approved by the Ethical Committee of Health Research of the Faculty of Medicine, Universitas Indonesia, Dr. Cipto Mangunkusumo General Hospital (Committee reference number: 821/UN2.F1/ETHIC/2017, approved on September 5, 2017).

Statistical analysis

Patient characteristics are presented descriptively. Normally distributed data are presented as the mean ± standard deviation, while abnormally distributed data are presented as the median, and categorical variables are presented as percentages. Pearson's correlation coefficient was used to evaluate the correlation between oocyte maturation rates, serum hCG levels, and LHR mRNA levels. Cutoff values were determined using the area under the receiver operating characteristic (ROC) curve. Data analyses were conducted using IBM SPSS Statistics for Windows, version 20.0 (IBM Corporation, Armonk, NY, USA).

RESULTS

Baseline patient characteristics and treatment outcomes are presented in Table 1. The median patient age was 35 years. Tubal factors were the most common causes of infertility. The women tended to be overweight with a mean BMI of 25.3 ± 4. The median serum hCG level, as determined the morning after hCG injection, was 87.2 mIU/mL. Serum hCG levels widely varied (range: 44.3–248.6 mIU/L). The percentage of mature oocytes was 68.2%, which was significantly associated with serum hCG levels in the morning after hCG injection.

## Table 1: Clinical and laboratory characteristics of the study participants

Variable	Value
Age (years), mean±SD	35.0±4.3
Causes of infertility, *n* (%)	
Tubal factors	7 (23.3)
Male factors	13 (43.3)
Unexplained	10 (33.3)
BMI (kg/m²), mean±SD	25.3±4.0
Anti-Müllerian hormone (ng/mL), mean±SD	2.7±2.1
Total doses of FSH (IU/L), median, minimum-maximum	2812 (1575-5550)
Estradiol serum (pg/mL), mean±SD	1778.8±1112.6
Total oocytes	336
Immature oocytes (%)	31.8
Maturation rate (%)	68.2
hCG level (mIU/mL), median, minimum-maximum)	87.2 (44.3-248.6)
LHR level (arbitrary unit)	4.9±2.9

SD=Standard deviation, BMI=Body mass index, FSH=Follicle-stimulating hormone, hCG=Human chorionic gonadotropin, luteinizing hormone receptor

When the patients were stratified by the percentage of mature oocytes, higher oocyte maturation was correlated with higher serum hCG levels at 12-h postinjection. At an hCG titer of 119.8 mIU/mL, all oocytes were mature, while at 60.5 mIU/mL, the maturation rate was <50%. LHR levels were approximately the same among the three stratified groups (~5.1 AU), while the maturation rates for Groups I, II, and III were 100%, 70%–99%, and 50%–69%, respectively. In group IV, the LHR level was only 3.5 AU, indicating that the maturation rate was <50% [Figure 1a and b]. The relationship between oocyte maturation rates and hCG and luteinizing hormone (LH) levels are shown in Figure 1c and d, respectively. Figure 2 was shown error bars between maturation rate with hCG level associated with Figure 1a. Further, Figure 3 was shown error bars between maturation rate with LHR expression associated with Figure 1b.

Serum hCG levels were positively correlated with the oocyte maturation rate ($r = 0.467$; $P < 0.01$), and a higher level of serum hCG at 12-h posttrigger was associated with a higher percentage of mature oocytes. Meanwhile, the rate of oocyte maturation was very weakly correlated with LHR expression of granulosa cells ($r = 0.073$; $P = 0.70$).

ROC analysis comparing maturation and serum hCG levels at 12-h posttrigger was conducted [Figure 4]. As shown in Figure 2, the cutoff value of serum hCG predictive of a high maturation rate was 77 mIU/mL, with an area under the ROC curve of 0.765 (95%
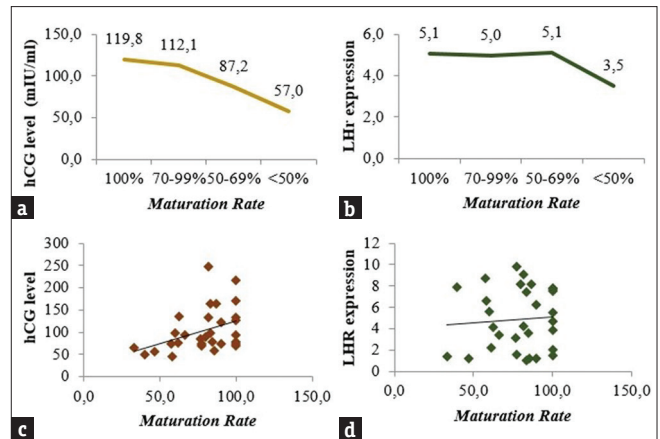
Figure 1: (a) Stratified maturation rate percentage group and human chorionic gonadotropin serum level, (b) stratified maturation rate percentage group and luteinizing hormone receptor expression, (c) trend line human chorionic gonadotropin level, and (d) trend line luteinizing hormone receptor expression
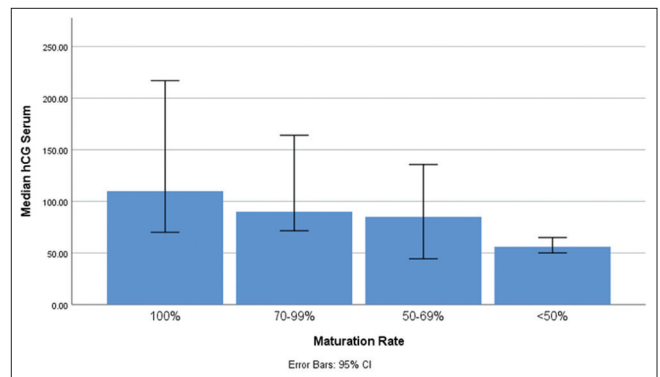

Figure 2: Bar error between maturation rate and human chorionic gonadotropin level
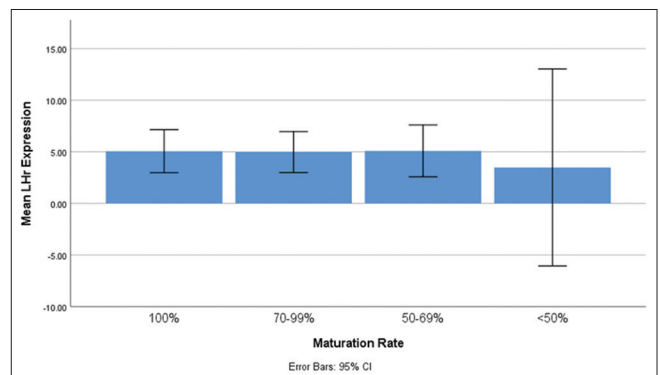
Figure 3: Bar error between maturation rate and luteinizing hormone receptor expression

confidence interval: 0.598–0.939), sensitivity of 78%, specificity of 67%, positive predictive value of 78%, and negative predictive value of 67% ($P < 0.001$). The mean maturation rate above the cutoff point was 85.5%, while below the cutoff point, the maturation rate decreased to 69% [Figure 5].
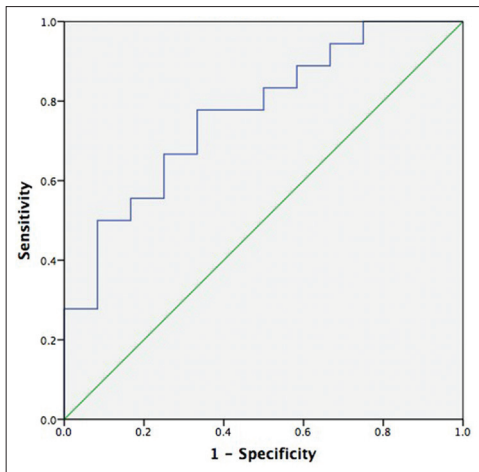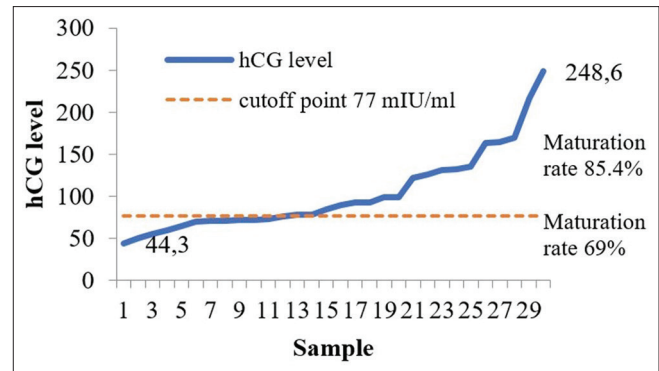
Figure 4: Receiver operator characteristic curve for the ability of serum human chorionic gonadotropin level to predict oocyte maturity. Area under the curve = 0.769.

Figure 5: The cutoff value of serum human chorionic gonadotropin level 12-h posttrigger with average oocytes maturation rate 85.5% in human chorionic gonadotropin level above cutoff value and 69% when human chorionic gonadotropin value below cutoff line

DISCUSSION

The results of the present study showed that serum hCG was moderately correlated with oocyte maturation. However, LHR mRNA expression was weakly correlated with oocyte maturation. Cutoff value for normal oocyte maturation of serum hCG was 77 mIU/mL ($r = 0.467$; $P = 0.009$).

Oocyte maturation is the continuation of meiosis to unfold metaphase II from arrested metaphase I. Follicles remain to develop until adolescence and arrest at the preovulatory stage. Further progress of meiosis I is started by LH-surge at which ovulation will begin.[2] Some studies have evaluated the correlation between oocyte maturity and patient age, as well as differences in controlled ovarian hyperstimulation dosing protocols, FSH levels, and the number of ovarian stimulation cycles. However, the correlation between serum hCG levels posttrigger and subsequent oocyte maturation remains unknown.[3-5] In this study, serum hCG was positively correlated with oocyte maturation. LH and hCG have the same structure of α-subunit and 85% of β-subunit. Therefore, hCG has the capability to induce oocyte maturation via LH receptor stimulation, granulose cells luteinization, and resumption of meiosis I. hCG also has greater affinity to stimulate LH receptor of human granulose cells. However, LH has the main impact to antiapoptotic proliferative signal inducing oocyte maturation and ovulation.[2]

In the present study, the data do not support a relationship between LHR expression of granulosa cells and the percentage of mature oocytes ($r = 0.073$; $P = 0.701$). In the follicular stage, the specific roles of LH in granulosa cells are not fully understood. FSH mainly regulates induction of LHR expression in granulosa cells, and as the follicle matures to the preovulatory stage, LHR induction increases in effectiveness.[6] Despite the importance of LH signaling of granulosa cells, the role of LHR on human follicular function has been studied to a limited extent, as current techniques are insufficient for adequate evaluation of LHR protein expression and activity. In particular, the level of LHR expressed on granulosa cells appears too low for accurate detection with currently available antibodies. Furthermore, measurement of the binding of radioactively labeled hCG does not provide adequate sensitivity to allow for LHR detection on naïve human granulosa cells in individual follicles. In contrast, *LHR* gene expression can be evaluated by qRT-PCR.[7] Moreover, monitoring of *LHR* gene expression is insufficient to determine whether the gene is translated into a functional receptor protein, which requires additional posttranslational modifications and proper localization to the cell surface in the mature form to be active.[8-12] Kishi *et al*. dictated that LHR expression mainly induced from FSH stimulation either directly or indirectly. Various factors enhance LHR expression, such as activin, insulin-like growth factor-1, estrogen, and interleukin-6. The other theory also states that LHR has a role to secrete inhibin-A-regulating granulosa cells luteinization. Overall, the precise functions of LH during the follicular phase of the human menstrual cycle remain unclear.[13]

The cutoff point of hCG for normal oocyte maturation was 77 mIU/mL. Therefore, at hCG serum levels below the limit, the maturation rate would be <75%. A low maturation rate is correlated with decreased fertilization and pregnancy rates.[14] Accordingly, the total hCG dosage to trigger optimal oocyte maturation rate should be adjusted to the hCG threshold. In contrast to the study conducted by Levy *et al*., there was no correlation between the oocyte maturation rate and hCG levels. Our study showed that a low level of hCG (<27 mIU/mL) still resulted in good oocyte maturation. Implicitly, this

is not related to the weight distribution of respondent which mainly have overweight. Many studies have evaluated the distribution of serum hCG is lower in obese patients due to larger volume. In other words, some patients may affect from standard dose of hCG, while the others may need an extra dose of hCG to stimulate the ovarium. However, the limitations of previous studies included a large variation in hCG dosages (5000–10.000 IU) to trigger oocyte maturation and time of serum hCG collection.[2]

There were some limitations to this study. The results of this study were not detail enough. More variables are needed to improve this study, such as fertilization rate, blastulation rate, transferred or frozen embryos, and pregnancy rate. This study also needs larger samples to enhance their accuracy.

Conclusion

Oocyte maturation had a correlation with a serum hCG with a cutoff value of 77 mIU/mL at the time of oocyte retrieval.

Conflicts of interest
There are no conflicts of interest.

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