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Twelve Hours Post-Injection Serum Human Chorionic Gonadotropin and Body Mass Index Predicts *In Vitro* Fertilization Oocyte Maturation Rate: A Cross-Sectional Study

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Abstract

Background: *In vitro* fertilization (IVF) remains a main treatment for infertility cases. Post-injection human chorionic gonadotropin (hCG) level is an essential factor in determining oocyte maturation rate in IVF. This study aimed to determine the relationship between 12 hours post-injection serum hCG level and oocyte maturation rate among IVF participants.

Materials and Methods: A cross-sectional study on IVF participants was done at a tertiary hospital in Indonesia from January 2020 to December 2021. Subjects were injected with 250 µg of recombinant-hCG (r-hCG) subcutaneously. Twelve hours post-injection serum hCG levels and oocyte maturation rate data were retrieved and analyzed accordingly.

Results: Twenty-eight subjects were recruited into the study. Higher 12 hours post-injection serum hCG was related to oocyte maturation rate (P=0.046). The cut-off point of 12 hours post-injection serum hCG to predict better oocyte maturation rate was 90.15 mIU/mL (sensitivity 68.2%, specificity 83.3%). Oocyte maturation rate may be predicted using body mass index (BMI) and 12 hours post-injection serum hCG.

Conclusion: Higher 12 hours post-injection serum hCG was associated with a higher oocyte maturation rate in IVF subjects.

Keywords: Human Chorionic Gonadotropin Hormone, *In Vitro* Fertilization, Oocyte, Oocyte Maturation

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Introduction

In vitro fertilization (IVF) is the most commonly performed procedure in assisted reproduction for achieving pregnancy (1). The mean pregnancy rate in IVF ranges from 30 to 35% (2). The success of IVF is closely related to various factors, most of which are still unknown until the later cycles of therapy or even near the end of treatment. The failure to achieve pregnancy is thought to be caused by embryo quality and endometrial receptivity factors. However, there have been no proven indicators to predict oocyte maturation rate during IVF (3, 4).

Before picking the ovum, controlled ovarian stimulation was carried out starting on the second day of the menstrual cycle. Following controlled stimulation, injection of human chorionic gonadotropin (hCG) is administered for the later stages of follicle maturation. Due to the nearly similar (homologous) chemical chain structure of hCG and luteinizing hormone (LH), hCG administration is aimed to mimic the normal LH surge in ovulation. The injection of exogenous hCG is usually done 12 hours before ovum pick-up (OPU). Previous studies had contrasting results linking serum hCG levels with oocyte quality and number at the time of OPU (5). However, there has been no research regarding the direct relationship between 12 hours post-injection serum hCG levels and oocyte maturity in the IVF cycle. This study

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aimed to determine the relationship between 12 hours post-injection serum hCG level and oocyte maturation rate among IVF participants.

Materials and Methods

An observational study with cross-sectional design was done to investigate the relationship between 12 hours post-injection serum hCG and oocyte maturation rate. The study was done at Yasmin Clinic, Cipto Mangunkusumo National Kencana Hospital, Jakarta, Indonesia, from January 2020 to December 2021.

Subjects recruited for the study were all 20-40 years old IVF participants who had hCG injection as part of the protocol and willing to participate in the study. Meanwhile, the exclusion criteria were subjects with history of ovarian surgery, chemotherapy, or radiotherapy. Poor responder subjects (defined by those with anti-Mullerian hormone level of <1.5 ng/mL) were also excluded from the study.

This study utilized the standard protocol of IVF applied in Cipto Mangunkusumo National General Hospital. A total of 250 µg recombinant hCG (r-hCG) was injected as exogenous hCG source on all subjects before OPU. Serum hCG level was obtained 12 hours post-injection in each subject. Moreover, the OPU was done 36 hours following hCG injection. Mature oocyte was defined as the oocyte reaching metaphase II according to the oocyte cumulus–corona complex (OCCC) grading, which included the `sun-burst' appearance of the corona radiata and expanded cumulus (6). Meanwhile, oocyte maturation rate was defined in this study as the rate of mature oocyte divided by the total oocyte picked up at OPU. The cut-off for satisfactory oocyte maturation rate was set to be 75% (7).

The Research Ethics Committee had approved the protocol for human studies with ethical clearance letter number KET-967/UN2.F1/ETIK/PPM.00.02/2019. All subjects had given their informed consent before their inclusion in the study. Collected data of the subjects were analyzed using Statistical Package for Socials Sciences (SPSS) for Macintosh ver. 20 (IBM®, USA). The characteristics of patients were analyzed descriptively. Normally distributed numeric variables were presented in the form of mean ± standard deviation, while abnormally distributed numeric variables were presented in the form of median (minimum value-maximum value). Bivariate analysis was done using unpaired t test for variables with normal distribution or Mann-Whitney test for variables with abnormal distribution. In order to determine the ideal cut-off of 12 hours post-injection serum hCG level, receiver operating characteristics (ROC) curve would be used with satisfactory oocyte maturation rate as the state variable. Afterwards, multivariate analysis consisting of 12 hours post-injection serum hCG level and clinical characteristics would be done using the logistic regression method.

Results

A total of 32 subjects fulfilled the inclusion criteria during the study. However, 4 subjects were excluded due to not taking serum hCG level examination. Therefore, only 28 subjects were recruited and analyzed. The study recruitment flowchart can be found in Figure 1.

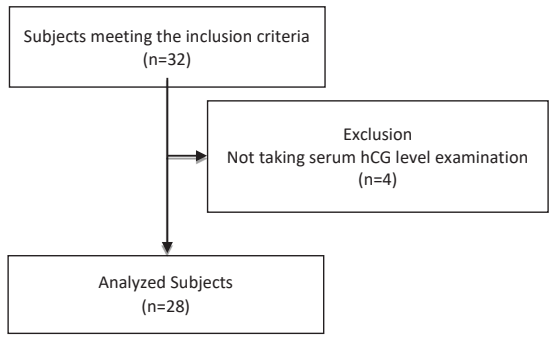

Fig.1: Recruitment flowchart of the study.

Following the recruitment of subjects, the subjects' clinical characteristics were analyzed. The results can be found in Table 1.

Table 1: Clinical characteristics of subjects

Characteristics	Frequency (n=28)
Age (Y)	33.32 ± 3.8
Body mass index (kg/m^2)	22.59 ± 2.9
Infertility duration (Y)	5.54 ± 2.7
Prior IVF history	
None	24 (85.7)
Once	2 (7.1)
Twice	2 (7.1)
Indication for IVF	
Infertility	27 (96.4)
Primary	24 (85.7)
Secondary	3 (10.7)
Social	1 (3.6)
Known etiology	
Male factors	9 (32.1)
Female factors	14 (50)
Uterine factor	10 (35.7)
Tubal factor	2 (7.1)
Ovarian factor	2 (7.1)
Female and male	2 (7.1)
Unknown	3 (10.7)
Basal AMH level (µg/mL)	3.29 (1.66-13.10)[#]
12 hours post-injection hCG level (mIU/mL)	101.81 ± 38.3
Oocyte maturation rate	
>75%	22 (78.6)
<75%	6 (21.4)
Oocyte retrieved per patient	11 (3-33)[#]

Data are presented as mean ± SD or n (%). IVF; *In vitro* fertilization, AMH; Anti mullerian hormone, hCG; Human chorionic gonadotropin, and [#]; Median (minimum value-maximum value).

Table 2: The relationship between 12 hours post-injection serum hCG level and oocyte maturation rate

Variable	Oocyte maturation rate	n	Mean ± SD	Mean diff	95% CI		P value
					Min	Max	
12 hours post-injection serum hCG level (mIU/mL)	≥75%	22	109.26 ± 38.0	34.78	0.60	68.95	0.046*
	<75%	6	74.48 ± 26.6				

hCG; Human chorionic gonadotropin, CI; Confidence interval, Min; Minimal, Max; Maximal, and *; Unpaired t test.

Table 3: Cut-off value for 12 hours post-injection serum hCG

AUC	12 hours post-injection serum hCG level (mIU/mL)	sn	sp	95% CI		P value*
				Min	Max	
78.8%	90.15	68.2	83.3	60.3%	94.0%	0.033

AUC; Aurea under the curve, Sn; Sensitivity, Sp; Specificity, CI; Confidence interval, Min; Minimal, Max; Maximal, and *; Unpaired t test

Furthermore, the analysis of relationship between 12 hours post-injection serum hCG level and oocyte maturation rate was also performed. The result of this analysis can be found in Table 2.

Based on the analysis, higher 12 hours post-injection serum hCG level was associated with a higher oocyte maturation rate (P=0.046). Moreover, the cut-off point for 12 hours post-injection serum hCG level was determined to predict a satisfactory oocyte maturation rate.

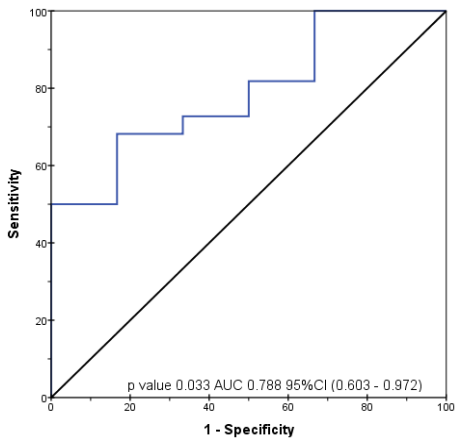

Fig.2: ROC curve of 12 hours post-injection serum hCG. ROC; Receiver operating characteristics, hCG; human chorionic gonadotropin, AUC; Aurea under the curve, and CI; Confidence interval.

Based on the analysis done in Table 3, it was found that the cut-off value for 12 hours post-injection serum hCG to predict satisfactory oocyte maturation rate was >90.15 mIU/mL. Furthermore, multivariate analysis was done. The result of the analysis can be found in Table 4.

Table 4: Multivariate analysis for predicting oocyte maturation rate

Variables	B	SE	Wald	P value	OR	95% CI	
						Min	Max
BMI	0.404	0.215	3.523	0.033	1.497	1.182	2.282
hCG	-0.032	0.018	3.140	0.048	1.012	1.005	1.030
Constant	-7.838	5.140	2.325	0.127			

BMI; Body mass index, hCG; Human chorionic gonadotropin, Min; Minimal, Max; Maximal, *; Logistic regression, B; Beta, S.E.; Standard of error, OR; Odds ratio, and CI; Confidence interval.

Based on the multivariate analysis, BMI and hCG level were the factors affecting oocyte maturation rate.

Discussion

Based on our analysis, higher 12 hours post-injection serum hCG was associated with higher oocyte maturation rate. The average age of subjects in our study was 33.32 ± 3.8 years, with most of the subjects having normal BMI. This value was similar to previous studies on IVF participants in Indonesia, with most subjects being aged over 30 years old (1, 2). In a previous study conducted in Jakarta, the best probability of IVF success was obtained when the participant's age was under 35 years old, with a success rate of 56.4% (1). Many factors are thought to be the cause of worse IVF success rate in older patients, some of which are disturbed hormonal factors and the existence of other comorbidities which increase along with aging (5). Other studies showed that subjects with older age would experience a higher rate of mitochondrial dysfunction associated with oxidative stress, resulting in worse oocyte quality (8). BMI is also known to be a determinant of oocyte maturity and clinical pregnancy in IVF through several mechanisms, one of which is lower hCG level in patients with higher BMI (1, 7). However, we could not generalize the result of this study as the number of included subjects in each body mass index group was not sufficient for adequate power in the analysis.

In this study, the level of oocyte maturity was assessed based on the rate of mature oocytes compared to the total number of oocytes obtained during oocyte pick up. The level of satisfactory oocyte maturation rate was determined to be more than 75%. Previous studies also used this value to predict clinical pregnancy in IVF patients (7, 8). In addition to this threshold, some studies determine other threshold values, depending on the agreement of the researchers (7, 9).

During the IVF procedure, hCG has a role to replace LH in triggering oocyte maturation in folliculogenesis. LH is required in the follicular phase to trigger paracrine signals between theca cells and granulosa cells, which are useful in triggering androgen and estrogen synthesis to achieve oocyte maturation. There are LH/choriogonadotropin (LH/CGR) receptors on the ovaries that can bind to both LH and hCG and play a major role in folliculogenesis. The role of hCG in folliculogenesis is largely related to its ability to bind to LH/CGR (10).

LH/CGR is required in the process of follicle maturation and ovulation. In the IVF procedure, hCG will replace the

role of the LH surge that should occur during ovulation by binding to the LH/CGR receptor. After the injection of r-hCG, it is estimated that a phenomenon similar to the LH surge will occur in a long time because the half-life is much longer, which is around 30 hours, so that a longer accumulation of effects will be obtained compared to physiological ovulation (10, 11).

Post-injection hCG levels in this study were assessed at 12 hours post-injection. Before the examination, all subjects were injected with r-hCG at a dose of 250 mg subcutaneously. This protocol was applied at the study site and was similar to the protocol used in the previous study (9). A previous study has shown that 250 mg r-hCG is superior than the 500 mg dose in preventing ovarian hyperstimulation syndrome (OHSS) (12). Although 12 hours post-injection hCG level in this study affected oocyte maturation, there were previous studies with contrasting results. One of the studies showing no correlation between hCG level and oocyte maturity nor embryo quality was the study performed by Gunnala et al. (13). However, Zhang et al. (14) recently stated that excessively high follicular fluid hCG levels would have a detrimental effect on oocyte maturation, fertilization, and embryonic development potential.

In addition to hCG levels, it was found that another factor influencing oocyte maturity in this study was body mass index. Previous studies indicated that IVF participants with higher body mass index are known to have worse oocyte maturation (1, 14). Previous studies suggested that larger doses of hCG should be injected in patients with a higher body mass index (12). However, this also carried a risk of increasing the likelihood of OHSS, so further research is needed to compare the benefits and impacts of the action (15, 16).

The main limitation of this study was its small sample size of the subjects. This limitation caused generalization to the general population unsuitable. Moreover, there was no data on the proportion of pregnancies, both biochemical, clinical, and pregnancy outcomes, so further analysis regarding the confounding factors of oocyte size could not be executed. The similarity of injected r-hCG dose among subjects also raised concerns due to body mass index being an influencing factors of oocyte maturity. Further studies with larger sample size and different r-hCG dose among the subjects may be considered.

Conclusion

Higher 12 hours post-injection serum hCG was associated with a higher oocyte maturation rate on IVF subjects. Furthermore, oocyte maturation rate might be predicted using BMI and 12 hours post-injection serum hCG.

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Authors' Contributions

B.W., E.S., V.S., A.K.H., G.P., K.S.; Conceptualization, Methodology, and Software. B.W., A.K.H., G.P., K.S., R.M., A.H.; Validation, Formal analysis, and Investigation. B.W., E.S., M.M., A.K.H., G.P., K.S.; Resources and Data Curation. E.S., B.W., M.M., A.K.H.: Writing- Reviewing and Editing. All authors read and approved the final manuscript.

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