

## Research Article

## Estradiol on Day Seven is a Good Predictor for Oocyte Maturation Rate in In Vitro Fertilization Program

### *Kadar estradiol hari ketujuh sebagai prediktor tingkat kematangan oosit pada program Fertilisasi In Vitro*

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#### Abstract

**Objective:** To determine which factors influence the rate of oocyte maturation in In Vitro Fertilization (IVF) program.

**Methods:** A retrospective cohort study was conducted using secondary data from IVF participants at the Yasmin Fertility Clinic, Dr. RSUP. Cipto Mangunkusumo, Jakarta, Indonesia during the period January 2019 to December 2020, as recorded in the InaRepromed archive. The variables analyzed were age, body mass index, and hormone levels on day 1, day 7, and day hCG, with oocyte maturation rate as the main outcome. Correlation test was performed between several variables and the level of oocyte maturation rate and followed by multivariate analysis to assess the factors that were closely related to oocyte maturation rate.

**Result:** Data from 52 subjects were collected for the study. Positive correlation was observed between oocyte maturation rate and estradiol on day 7 ( $r = 0.229$ ), while negative correlation was observed between oocyte maturation rate and progesterone/estradiol ratio on day 7 ( $r = -0.289$ ) and luteinizing hormone on day 1 ( $r = -0.265$ ). Multivariate analysis revealed that higher estradiol on day-7 was associated with better oocyte maturation rate ( $p = 0.047$ ).

**Conclusion:** Higher estradiol level on day 7 was associated with better oocyte maturation rate in IVF.

**Keywords:** Assisted reproductive technology (ART), estradiol, in vitro fertilization (IVF), progesterone.

#### Abstrak

**Tujuan:** Untuk menentukan faktor-faktor yang mempengaruhi tingkat pematangan oosit dalam program Fertilisasi In Vitro (FIV).

**Metode:** Desain penelitian adalah kohort retrospektif, menggunakan data sekunder peserta bayi tabung Klinik Fertilitas Yasmin, RSUP Dr. Cipto Mangunkusumo, Jakarta, Indonesia selama periode Januari 2019 hingga Desember 2020, yang tercatat di dalam arsip InaRepromed. Variabel yang dianalisis adalah umur, indeks massa tubuh, dan kadar hormon pada hari ke-1, hari ke-7, dan hari ke-hCG, dengan tingkat maturasi oosit sebagai luaran utama. Dilakukan analisis korelasi antara beberapa variabel dengan tingkat maturasi oosit, dan dilanjutkan dengan analisis multivariat untuk menilai faktor-faktor yang berhubungan kuat dengan tingkat maturasi oosit.

**Hasil:** Data dari 52 subjek dikumpulkan untuk penelitian ini. Dijumpai korelasi positif antara tingkat maturasi oosit dan estradiol pada hari ke 7 ( $r = 0,229$ ), sedangkan korelasi negatif diamati pula antara tingkat maturasi oosit dan rasio progesteron/estradiol pada hari ke 7 ( $r = -0,289$ ) dan hormon luteinisasi pada hari 1 ( $r = -0,265$ ). Analisis multivariat mengungkapkan bahwa estradiol yang lebih tinggi pada hari ke-7 dikaitkan dengan tingkat maturasi oosit yang lebih baik ( $p = 0,047$ ).

**Kesimpulan:** Kadar estradiol yang lebih tinggi pada hari ke 7 dikaitkan dengan tingkat pematangan oosit yang lebih baik pada program FIV.

**Kata kunci:** Teknologi Reproduksi Berbantu (TRB), estradiol, Fertilisasi In Vitro (IVF), progesteron.

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## INTRODUCTION

Infertility is categorized as a disease by the World Health Organization, in the form of disability in partners who are regularly sexually active and do not use contraception to achieve clinical pregnancy after 12 months (<35 years) or 6 months (>35 years).<sup>1,2</sup> Epidemiologically, infertility is experienced by 15% of all couples of different sex globally.<sup>2</sup> As a problem including couples, infertility may be caused by either female and male. Therefore, a thorough evaluation of both husband and wife is needed.<sup>1,2</sup>

There are several methods of treating infertility, one of which is the assisted reproductive technology (ART) program as a treatment for infertility caused by both male factor and female factor.<sup>2,3</sup> ART might be considered as one of the options in some cases, such as uncorrected tubal occlusion, moderate endometriosis, sperm factors, or repeated failure of intrauterine insemination.<sup>3</sup> The philosophy of ART is to grow several oocytes simultaneously, which start at the early follicular phase so that some of the collected mature eggs can be fertilized with spermatozoa. This technology requires a controlled ovarian hyperstimulation (COH) approach.

The quality of oocytes in the ART program is one of the most important things in predicting the success of the ART program.<sup>4</sup> There are several oocytes produced in the ART program through a controlled ovarian hyperstimulation process. Some of these oocytes are retrieved together, fertilized, and cultured until they become embryos which will be returned eventually to the mother's endometrial cavity.<sup>5</sup> The maturation level of oocytes is one of the factors affecting quality of oocytes. The success of ART generally depends on three main factors, namely oocyte quality, sperm quality, and uterine quality.<sup>4,5</sup> These three factors will be influenced by various characteristics of the patient, such as genetics, age, body mass index, and other comorbidities.<sup>6</sup>

Ovarian reserve and the sensitivity of the follicular response to growth after COH are factors that strongly influence the oocyte maturation rate, and this condition can be reflected in the reproductive hormones levels of each participant in the ART program. This study aimed to determine which factors associated with oocyte maturation rate on ART participants.

## METHODS

This is a retrospective cohort study, conducted using secondary data from IVF participants in Yasmin Fertility Clinic, Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia during the period of January 2019 to December 2020. Data were collected from the Ina-Repromed archive.

This study used 5% error bound and 95% confidence interval limit, with power of the test considered to be 90%. This study was conducted at Yasmin Clinic, dr. Cipto Mangunkusumo National General Hospital, Jakarta, Indonesia on January 2019 to December 2020. The inclusion criteria for this research were all ART participants aged 30 to 45 years old and having undergone in vitro fertilization (IVF) program using frozen embryo transfer (FET) or intracytoplasmic sperm injection (ICSI), with antagonist protocol. Subjects with incomplete medical record data were excluded from the study.

Variables analyzed in this study were age, body mass index, day 1; anti-mullerian hormone (AMH), follicle stimulating hormone (FSH), luteinizing hormone (LH), day 7; estradiol, progesterone, progesterone-to-estradiol ratio, and day hCG; estradiol, progesterone, progesterone-to-estradiol ratio, FSH, LH level.

All human studies had been approved by the Research Ethics Committee of Faculty of Medicine, Universitas of Indonesia. All patients who were included in this study had given the informed consent prior to the study.

Collected data were then analyzed using SPSS for Macintosh ver. 20. Characteristics of subjects and examination results were analyzed descriptively. Correlation between variables and oocyte maturation rate was calculated. Multivariable analysis was done in order to determine factors associated with oocyte maturation rate.

## RESULTS

On the course of this study, a total of 52 subjects were included in this study. Baseline characteristics of subjects can be found on Table 1. Based on the analysis, it was found that the average age of subjects were 37 years old with normal body mass index, and having hormonal level within normal limit.

**Table 1.** Characteristics of Subjects

Characteristics	Characteristics
Age (years)	36.93 + 4.31
Body mass index (kg/m <sup>2</sup> )	24.35 (16.1 – 37.7)
AMH day 1 (ng/mL)	3.09 (0.02 – 18.9)
FSH day 1 (mIU/mL)	8.32 (1.90 – 17.4)
LH day 1 (mIU/mL)	4.55 (1.32 – 18.47)
Estradiol day 7 (pg/mL)	123.1 (9.98 – 40.0)
Estradiol day hCG (pg/mL)	1.763 (619 -5.187)
Progesterone day 7 (ng/mL)	0.58 (0.1 – 1.98)
Progesterone day hCG (ng/mL)	0.84 (0.3 – 5.5)
Progesterone-to-estrogen ratio day hCG	0.51 (0.18 – 2.71)
Mature oocyte number	5 (1 – 29)
Total oocyte number	7 (1 – 32)
Oocyte maturation rate	0.79 (0.1 – 1)

All of the variables in this study were analyzed for correlation with oocyte maturation rate. Based on the analysis, it was found that positive correlation was observed between oocyte maturation rate and estradiol on day 7 ( $r = 0.229$ ), while negative correlation was observed between oocyte maturation rate and progesterone/estradiol ratio on day 7 ( $r = -0.289$ ) and luteinizing hormone on day 1 ( $r = -0.265$ ). The result of this analysis can be found on Table 2.

**Table 3.** Multivariable Analysis Result: Step 2

Variables	Unstandardized Coefficients		Standardized Coefficients		
	B	Standard Error	Beta	T	P-value
Estradiol day 7	0.001	0.000	-0.306	2.267	0.028
Progesterone day hCG	-0.049	0.030	-0.216	-1.598	0.116
Constant	0.755	0.054	-	14.029	0.000

**Table 4.** Multivariable Analysis Result: Step 2

Variables	Unstandardized Coefficients		Standardized Coefficients		
	B	Standard Error	Beta	T	P-value
Estradiol day 7	0.001	0.000	0.270	2.041	0.047
Constant	0.710	0.046	-	15.266	0.000

## DISCUSSION

In this study, it was found that the variable that can be used to predict the oocyte maturation index of subjects with controlled ovarian hyperstimulation (COH) is estradiol on day 7. Although one of the previous multivariate analysis steps had shown the effect of progesterone on the hCG day on the oocyte maturation rate, the last step of the multivariate analysis only showed that estradiol on day 7 was the dominant factor to predict the oocyte maturation index in COH.

Estradiol level is one of the parameters that can determine the aging of follicular cells, because

**Table 2.** Correlation between variables and oocyte maturation rate

Variables	Oocyte Maturation Rate	
	r	P-value
Age (years)	-0.103	0.469
Body mass index (kg/m <sup>2</sup> )	-0.187	0.185
AMH day 1 (ng/mL)	-0.165	0.244
Estradiol day 7 (pg/mL)	0.229	0.102
Progesterone day 7 (ng/mL)	-0.111	0.434
Progesterone-to-estrogen ratio day 7	-0.289	0.038
FSH day 1 (mIU/mL)	0.173	0.221
LH day 1 (mIU/mL)	-0.265	0.057
Estradiol day hCG	-0.112	0.430
Progesterone day hCG	-0.189	0.179
Progesterone-to-estrogen ratio day hCG	-0.056	0.694
FSH day hCG (mIU/mL)	-0.138	0.331
LH day hCG (mIU/mL)	-0.052	0.715

Additionally, multivariable analysis revealed that higher estradiol on day-7 was associated with better oocyte maturation rate ( $p = 0.047$ ). The result of this analysis can be found on Table 3 and 4.

granulosa cells are the main source of follicular estrogen (E2 - estradiol). Increased levels of estrogen are associated with follicular cells that are getting more mature or experiencing atresia. Therefore, estrogen might be used as one of the parameters of the proportion of maturation.<sup>7</sup>

Research conducted on sheep oocytes found that estradiol levels were directly related to the stimulation of oocyte maturation.<sup>8</sup> Other studies on in vitro fertilization of bull and ewe oocytes found that oocytes having richer estradiol environment will have higher nucleus maturation rate than oocytes with low environmental estradiol levels.<sup>9</sup> However, excessive concentration of estradiol

would actually inhibit oocyte maturation.<sup>10</sup>

The results showed that higher serum estradiol level in women undergoing assisted reproductive technology programs would not inhibit the maturation level of oocytes, but directly increase the number of mature oocytes obtained, thereby increasing the maturation level of oocytes.<sup>13</sup> The pathophysiology underlying more mature oocytes in ART participants who have higher estradiol levels is based on the growth and development of dominant follicles. The growth of dominant follicles will cause the secretion of estradiol and inhibin A. This secretion indicates better cytoplasmic maturation and more advanced folliculogenesis.<sup>9</sup>

In this study, it was also known that progesterone level did not affect oocyte maturation rate. Previous studies has shown that progesterone level in ART program participants was one of the variables that have been widely studied to affect the number of oocytes obtained, the maturation level of the oocytes, the fertilization rate, and the success of pregnancy. This phenomenon is associated with the incidence of premature luteinization.<sup>11,12</sup> Premature luteinization is a term used to refer to a premature LH surge (LH surge), with serum progesterone levels being used as an indication of premature luteinization in various studies.<sup>11,12</sup>

### CONCLUSIONS

It is concluded in this study that higher estradiol level on day 7 was associated with better oocyte maturation rate on ART participants with COH.

### DECLARATIONS

The data used in this study can be requested from corresponding author upon reasonable request.

### CONFLICT of INTEREST

Authors declare that there is no conflict of interest in this study.

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