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The Relationship of Antral Follicle Count (AFC) with Levels of Estradiol Serum and Mature Ovarian Follicles in Women Undergoing the IVF Program at the Graha Amerta Fertility Clinic of General Hospital of Dr. Soetomo in the Period 2018

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Abstract

Background: In the last two decades, one of the critical factors of assisted reproductive technology (ART) has been identified as ovarian capacity. Consequently, ovarian capacity markers have evolved to be part of diagnostic testing conducted regularly before IVF. Several predictor factors, such as antral follicle count (AFC), is one of several factors to predict ovarian capacity. These diagnostic procedures and tests have the purpose of identifying women at high-risk patient who respond poorly toward controlled ovarian stimulation throughout the IVF cycle. The ovarian response toward ovulation stimulation within this research was assessed by peak estradiol (E2) serum levels and the total number of mature follicles. There is no clear relationship between AFC and levels of estradiol serum and the number of mature ovarian follicles to identify women who respond poorly. **Methods:** This study is an analytical study with a cross-sectional design. Samples were taken from secondary data of medical records using the total sampling method on women undergoing the IVF program at the Graha Amerta fertility clinic of General Hospital of Dr. Soetomo from January 1 to December 31 2018 according to the inclusion and exclusion criteria. The AFC variables, estradiol serum levels, and the number of mature ovarian follicles in the study were analyzed using Spearman's correlation. **Conclusion:** There is a significant relationship between antral follicle count (AFC) with the levels of estradiol serum and the number of mature follicles in women undergoing the IVF program.

Keywords: Antral follicle count (AFC), estradiol (E2) serum levels, the number of mature ovarian follicles

Introduction

Infertility considered as one of the global public health problems. Nowadays, infertility is an event that must be paid attention to because, corresponding to the World Health Organization, infertility in women currently being the severe fifth-highest incident in the

global scope. Currently, the highest pregnancy success rate achieved in the world by the IVF program has only reached 20%.^{1,2} The rapidly developing medical science and technology answers the issue of difficulties in having offspring through Assisted Reproductive Technology (ART), such as In Vitro Fertilization (IVF).³ Over the last twenty years, one of the critical factors of assisted reproductive technology (ART) has been identified as ovarian capacity. Consequently, ovarian capacity markers have been considered to be included in the routine diagnostic testing performed before IVF.⁴

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Based on several studies, ovarian capacity can be predicted by several predictor factors, including ovarian volume, antral follicle count (AFC), ovarian stromal blood flow, and hormonal markers such as follicle-stimulating hormone (FSH), estradiol (E2), anti-Mullerian hormone (AMH), and inhibin B.4 These diagnostic procedures and tests have the purpose of identifying women at high-risk patient who respond poorly toward controlled ovarian stimulation throughout the IVF cycle.5 The ovarian response to ovulation stimulation can be assessed by the total number of mature follicles, the total number of oocytes obtained in the ovum pick-up procedure, the number of mature oocytes obtained in the ovum pick-up procedure, and the total number of gonadotropin doses needed in the ovulation stimulation cycle.4

According to an article, some recent studies have shown that AFC is a useful predictor of ovarian response to ovulation induction drugs.6 Moreover, AFC is considered to be the best single predictor of ovarian response in the ARB cycle. There is no clear relationship between antral follicle count and levels of estradiol serum and the number of mature ovarian follicles.

Based on the description above, it is necessary to conduct a study on "the relationship between antral follicle count (AFC) and levels of estradiol serum and the number of mature ovarian follicles in women undergoing the IVF program" with the hope of providing scientific information for later use in the initial evaluation of infertile patients in the procedures of IVF program. In the future, the success rate of IVF for infertile couples can increase in Surabaya and at the national and global levels.

Materials and Methods

Experimental Design

This research has been approved by Health Research Ethics Committee RSUD Dr. Soetomo Surabaya with ethical clearance number 1521/KEPK/IX/2019. It was an analytical study with a cross-sectional design. Samples were taken from secondary data of medical records on women undergoing the IVF program at the Graha Amerta fertility clinic of General Hospital of Dr.

Soetomo from the period of January 1 to December 31 2018 according to the inclusion and exclusion criteria.

Data was collected using the total sampling method. The data taken included age, occupation, residence, levels of E2 serum on day 3, levels of FSH serum on day 3, levels of LH serum on day 3, AFC, E2 serum levels before OPU, number of mature follicles, and type of ovulation stimulation drugs. Exclusion criteria were female patients with ovulation disorder Polycystic Ovary Syndrome (PCOS), endometriosis, and history of ovarian surgery. Incomplete data were excluded from the study.

Statistical Analysis

The data obtained were then analyzed using observational analysis methods, processed using the SPSS v 25.0 program. The Kolmogorov Smirnov normality test was performed on research variables and with Spearman's correlation analysis and presented descriptively. P values less than 0.05 were considered statistically significant.

Result

A total of 209 couples participated in the IVF program in the 2018 period. The results of medical record data that met the inclusion criteria were 126 samples. Most of the respondents were women of productive age with a mean of 34.63 ± 4.714 years, and the largest age group was 30 - 34 years as many as 48 (38.1%). Based on occupation, most private employees (31.4%) followed by housewives (18.6%). In the category of self-employed and other groups, respectively 17, 1%. Other groups work as lecturers, teachers, doctors, midwives, honorary, and the Indonesian Navy. In addition, the majority of patients (99; 78.6%) resided in East Java. The data result (Table 1) showing the lowest AFC of 2 and the highest of 20 with a mean of 8.17 ± 3.64 calculated from both right and left ovaries. The lowest level of E2 serum before OPU in patients was 14.80 and the highest of that was >3000 pg/mL with a mean of 1838.53 ± 876.28 pg/mL. The lowest and the highest number of mature follicles were 0 and 19 respectively, with a mean of 6.21 ± 3.78 .

Tabel 1. Descriptive analysis of AFC, levels of E2, and the number of mature ovarian follicles in women undergoing IVF at the Graha Amerta fertility clinic in 2018

Description	Total		Descriptive	
	n	%	Min - Max	Mean (SD)
AFC	126	100	2 – 20	8.17 (3.64)
Levels of E2 Serum			14.80 – 3001	1838.53 (876.28)
The number of mature follicles			0 – 19	6.21 (3.78)

The analysis of AFC based on the respondents' age (Table 2) showed that the maximum number of AFC in the age category of 20-24 years was 12 with a mean of 9.33 ± 2.517 . In the age category of 25-29 years, the lowest AFC was 5 and the highest was 15 with a mean of 9.38 ± 3.070 . The ages of 30-34 years had the lowest AFC of 4 and the highest AFC of 20 with a mean of 9.00 ± 3.345 . The lowest AFC in the age category of 35 - 39 years was 2 and the highest AFC in this category was 20 with a mean of 8.57 ± 3.788 . In the age category of 40 - 45 years, the mean AFC was the lowest compared to other age categories, in which the lowest AFC was 2 and the highest AFC was 8 with a mean of 4.35 ± 1.872

Table 2. Age-based AFC analysis of women undergoing IVF at the Graha Amerta fertility clinic in 2018

Age (years)	Total (n=126)	AFC	
		Min - Max	Mean (SD)
20 – 24	3	7 – 12	9.33 (2.517)
25 – 29	13	5 – 15	9.38 (3.070)
30 – 34	48	4 – 20	9.00 (3.345)
35 – 39	42	2 – 20	8.57 (3.788)
40 – 45	20	2 – 8	4.35 (1.872)

Data of AFC, levels of E2 serum before ovum pick-up (OPU), and the number of mature ovarian follicles were tested for normality of data with the Kolmogorov Smirnov test with $p=0.002$; 0.002 ; 0.000 respectively, indicating that the data was not normally distributed. Therefore, Spearman's correlation method is used.

Based on the statistical tests results on 50 samples using the Spearman method, the r-value was 0.526, indicating a moderate correlation between AFC and

levels of E2 serum stimulated by FSH. It was found that the $p=0.000$ value was smaller than 0.05. Therefore, it can be concluded that the moderate correlation was statistically significant. In the group that received a combination of FSH - LH of a total of 76 samples (Table 3), the results of the Spearman's correlation test showed a moderate and significant correlation between the variables of AFC and levels of E2 serum with combined FSH - LH ovulation stimulation ($r=0.571$; $p=0.000$).

Table 3. Relationship between AFC and levels of E2 serum before OPU based on the stimulation of FSH and a combination of FSH & LH

Variable	Type of Ovulation Stimulation Drugs	Total (n)	r-Value (correlation coefficient)	p-Value (sig)
AFC àLevels of E2 serum before OPU	FSH	50	0.526**	0.000
	Combination of FSH & LH	76	0.571**	0.000

The results of statistical tests on 50 samples using the Spearman method show that the r-value was 0.603, indicating a moderate correlation between AFC and the number of mature follicles stimulated by FSH. Besides, it was found that the p=0.000 value was smaller than 0.05, so that it can be concluded that the correlation was statistically significant. Patients with combination stimulation of FSH - LH show an r-value of 0.480, indicating that AFC correlated with the number of mature follicles (Table 4). This relationship was statistically significant (p=0.000).

Table 4. Relationship between AFC and Maturity Follicle Count based on stimulation of FSH and a combination of FSH & LH

Variable	Type of Ovulation Stimulation Drugs	Total (n)	r-Value (correlation coefficient)	p-Value (sig)
AFC àNumber of mature follicles	FSH	50	0.603**	0.000
	Kombinasi FSH & LH	76	0.480**	0.000

Discussion

The data from this study follows the ²⁸ Human Fertilization and Embryology Authority or HFEA report that stated the characteristics of patients who participated in the FIV program in 2016 in the UK were patients aged less than 35 with an average of 35.5 years.⁷ This is also following previous research that was conducted at the Graha Tunjung IVF Clinic at Sanglah Hospital, the ²⁹ study found that most patients in the IVF program were in the age group less than 35 years old.⁸

According to a research on the characteristics of infertile pairs shows that the most respondents' occupation is private workers (47%).⁹ Another study that discusses the factors that affect infertility in women stated that most infertile women are women who work in the type of work most found are private workers.¹⁰ The

results of this study related to respondent's occupation are in line with another research about infertility, which states that working women (career women) have an increased risk of infertility 3.91 times greater than women with normal stress levels and statistically significant (p = 0.046).¹¹

³¹ Ovarian aging appears to play a major part in reproductive aging and is associated with decreased oocyte quality and slowly decrease in the number of primordial follicles, which will be reduced exponentially through childhood and adulthood, resulting in nearly follicle-free ovaries at menopause. Likewise, the number of follicles that has entered during the growth phase through the ³² antral development stage falls with age. A decrease in ovarian volume and the number of antral follicles are characteristics of ovarian aging. According to a study, AFC is a significant parameter compared to

patient age and other endocrine markers in indicating an ovarian reaction towards stimulation that has been given in advance.¹²

¹³ Demographic and clinical studies show that female fertility begins to subside from the age of 30, and the ability to get pregnant has become nearly zero at an average age of 41 years.¹³ The outcome of this study shows that in the age category of 40 - 45, the average AFC was the lowest compared to other age categories, in which the lowest AFC was 2, and the highest was 8 with a mean of 4.35 ± 1.872 . The older the women undergoing the IVF program at the Graha Amerta fertility clinic, the lower the average AFC. Another research stated that AFC could help determine the stimulation protocol; based on some studies, it has been proven to be the most calculable determinant of oocyte retrieval per initial FSH dose.¹⁴

All research subjects in this study were IVF participants using the GnRH antagonist protocol. Ovarian stimulation was begun on the third day using recombinant FSH drugs, and then the injection was controlled following each of the participant's reactions towards the dose given. Gonadotropin-releasing hormone (GnRH) antagonist administered to deny premature LH surge begins at the time follicle attained 14 mm in diameter.¹⁵ Another research stated that GnRH antagonists' use in ovarian stimulation protocols had provided a new option in the promotion of multifollicular growth.¹⁶ Antagonists cause direct suppression in releasing gonadotropin, in the absence of the flare-up effect. FSH is considered to play a more critical role in multifollicular growth. However, it is possible to suggest that LH can be advised to be included in all ovulation induction protocols, since it is difficult to ascertain which individual will be advantaged from LH administration. Currently, no evidence has been made regarding the negative effects of LH to the ovarian stimulation outcome.

According to the results of the statistical tests on 50 samples using the Spearman method, the r-value was 0.526, indicating a moderate correlation between AFC and levels of E2 serum stimulated by FSH. Besides, it was found that the $p=0.000$ value was smaller than 0.05, so it can be concluded that the moderate correlation was statistically significant. Meanwhile, in the group that received a combination of FSH - LH of 76 samples, Spearman's correlation test results show a moderate and significant correlation between the variables of AFC and

levels of E2 serum with combined ovarian stimulation of FSH - LH ($r=0.571$; $p=0.000$). The same results as this study were found in research that examined the relationship of AFC among various ovarian examination parameters, including levels of E2 serum.¹⁷ The results show that AFC was significantly associated with levels of E2 serum before OPU. Another research about the AFC application in clinical settings to indicate whether AFC acquired from various observers is a predictor of the quality of the stimulation with the parameter of peak levels of E2 serum, scientifically showing a major positive correlation between AFC and peak levels of E2 serum.⁶

The statistical tests on 50 samples using the Spearman method show that the r-value was 0.603, indicating a moderate correlation between AFC and the number of mature follicles stimulated by FSH. Besides, it was found that the $p=0.000$ value was smaller than 0.05, so it can be concluded that the correlation was statistically significant. Patients with combination stimulation of FSH - LH show an r-value of 0.480, indicating that the number of mature follicles corresponds to the AFC. This relationship was statistically significant ($p=0.000$). It is in line with the results of another study, stating that there was a significant relationship between AFC and ovarian response to ovarian stimulation.⁴ In this study, the ovarian response was examined by four parameters, such as the quantity of mature follicles. The higher the number of AFC, the higher the number of mature follicles.

² Poor ovarian response was first described in 1983 as having a peak estradiol concentration <300 pg/mL and inadequate follicular production resulting in fewer eggs being acquired, thus declines the amount of embryos transferred. In that sense, a poor ovarian response leads to drop of oocyte production, cycle cancellation, and overall is corresponded with a significantly reduced chance of pregnancy.²⁰ Data presented by another study proved that AFC provides more significant prediction regarding the incident of a poor response to hormone stimulation in IVF compared to the patients' age and other examination that is recently used, for example, basal FSH, E2, or inhibin B.¹⁹ It can be stated that AFC becomes a good prognosis of poor ovarian response. It is an essential evaluation in an IVF program because a patient undergoing an IVF program due to a poor ovarian response is generally regarded to have a lower pregnancy rate than those with a normal ovarian response.

Conclusion

1. There is a relationship between antral follicle count (AFC) and estradiol (E2) serum levels in women undergoing the IVF program at the Graha Amerta fertility clinic at General Hospital of Dr. Soetomo for the period 2018.

2. There is a relationship between antral follicle count (AFC) and the number of mature follicles in women undergoing the IVF program at the Graha Amerta fertility clinic at General Hospital of Dr. Soetomo Hospital for the period 2018.

Conflict of Interest: The authors declare that there is no conflict of interest.

Ethical Clearance: This research has been approved by Health Research Ethics Committee RSUD Dr. Soetomo Surabaya with ethical clearance number 1521/KEPK/IX/2019.

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