Correlation between follicular fluid AMH levels and numbers of oocytes in polycystic ovarian syndrome patients undergoing in vitro fertilization

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Abstract. Anti-Mullerian Hormones (AMH) are glycoproteins secreted by the granulosa cells of small antral and pre-antral follicles for the regulation of early follicular development. Impaired folliculogenesis may result in excess accumulation of follicles resulting in increased levels of AMH associated with polycystic ovarian syndrome (PCOS). The numbers of studies on the relationship between the follicular fluid AMH levels and the number of oocytes are limited. In this study, we investigated the correlation between the AMH levels in follicular fluid and the numbers of oocytes in patients with PCOS undergoing in vitro fertilization (IVF). This cross-sectional study was conducted at Yasmin IVF Clinic, Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia, between November 2016 and March 2017. Follicular fluid from 20 patients with PCOS aged 27–39 years and who were undergoing IVF were aspirated under transvaginal ultrasound guidance, and their AMH levels were measured using enzyme-linked immunosorbent assay. The correlation between the follicular fluid AMH levels and the number of oocytes was analyzed using Spearman correlation. Increase in the AMH levels in the follicular fluid of patients did not affect the number of oocytes detected (p = 0.862), with a mean AMH level of 5.49 ng/mL associated with 18 oocytes. Despite the lack of statistical significance, the correlation graphic demonstrated a trend of increasing AMH levels with a decreasing number of oocytes (r = −0.041). No correlation was noted between the level of AMH in the follicular fluid and the number of oocytes in the study patients with PCOS. Analysis of a larger sample size is warranted to confirm the current data.

1. Introduction
Polycystic ovarian syndrome (PCOS) is the most frequent cause of anovulatory infertility and hyperandrogenism in young women [1], which affects up to 10% of all reproductive-age women
in the world [2]. PCOS is characterized by a clustering of hyperandrogenism, hyperinsulinemia, hypersecretion of luteinizing hormone (LH), menstrual dysfunction, hirsutism, infertility, and pregnancy and neonatal complications [3]. Although patients with PCOS typically produce a large number of oocytes, the oocytes are often of poor quality, which leads to reduced fertilization and cleavage and implantation rates and a higher rate of miscarriage in patients with PCOS undergoing *in vitro* fertilization (IVF) treatment [4].

Anti-Mullerian hormone (AMH) is a glycoprotein of the transforming growth factor-beta (TGF-β) superfamily. AMH is produced by granulosa cells from 36 weeks of gestation until reaching menopause, with the highest expression occurring in small antral follicles. The rate of AMH production gradually declines as follicles grow, and once the follicles reach a size at which they are dominant, AMH mostly disappears. AMH disappearance from these larger follicles appears to be an important requirement for dominant follicle selection and progression to ovulation because AMH plays an inhibitory role in the ovary and reduces both primordial follicle initiation and follicle sensitivity to follicle-stimulating hormone (FSH) by the inhibition of aromatase. Because of these reasons, AMH is a focus of interest in PCOS [5].

The serum and follicular fluid (FF) AMH levels are elevated in women with PCOS compared with controls [6]. A recent study suggested that increased FF AMH levels in women with PCOS may have harmful consequences on oocyte quality and maturation via an unclear molecular mechanism, but also that it does not have any effect on the pregnancy rates [7]. To determine whether AMH contributes to the success of IVF-embryo transfer, this study aimed to confirm the relationship between the FF AMH levels and its correlation with the numbers of oocyte in patients with PCOS undergoing IVF.

2. Methods

The study protocol was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia-Cipto Mangunkusumo Hospital. This study was a cross-sectional analysis of subjects fulfilling the following criteria:

2.1. Patient Eligibility

The eligibility criteria for the study subjects were as follows: women who had undergone IVF and were diagnosed with PCOS based on their AMH level. Also, SOPK diagnosis-based Rotterdam criteria (at least two of the three following criteria):

(1) Ovulatory disturbance, mainly oligomenorrhea or amenorrhea
(2) Hyperandrogenism, defined either clinically by hirsutism (modified Ferriman and Gallwey score > 6) or severe acne/seborrhea and/or defined biologically by a testosterone serum level > 0.7 ng/mL and/or D4-androstenedione level > 2.2 ng/mL
(3) More than 12 follicles measuring 2–9 mm in each ovary, with a peripheral distribution noted on ultrasound and/or an ovarian volume of >10 mL. Ultrasound examination was performed with a 7.5 MHz transvaginal transducer.

The inclusion criteria for control included women with infertility dysfunction caused by factors other than ovarian issues and women who signed the informed consent form.

Exclusion criteria for the study subjects included women with endometriosis disease or ovarian dysfunction other than PCOS, those who smoked actively, those who used hormonal contraception in a stimulation cycle, and those who declined to sign the informed consent form.

2.2. Place and Time of Study

The study was conducted between November 2016 and March 2017 at the Yasmin Clinic, Dr. Cipto Mangunkusumo Public Hospital and at the Daya Medika Clinic, Jakarta.
2.3. Sample Assessment
Sample selection was based on the inclusion and exclusion criteria. Samples were selected on the basis of anamnesis, physical examination (general status and obstetrics status), and the findings of ultrasonography and Rotterdam criteria laboratory examination. Potential patients were asked to submit their informed consent and agreement for participation in the study. The patients were examined on the basis of the standard operating procedures at the Yasmin Clinic. FF was collected by ovum pick-up procedure.

2.4. ELISA
FF was analyzed for calculating the AMH level. Assessment was conducted using the AMH Gen II ELISA Kit (Beckman Coulter Inc., USA). Calibrator, control, and sample were incubated in microtitration and then coated with anti-AMH antibody. Next, incubation and washing were conducted. Antibodies that detected anti-AMH were labeled with biotin and added to each well, followed by another round of incubation and washing. Then, streptavidin–horseradish peroxidase (HRP) was added to each well. Incubation and washing were conducted yet again. After this, tetramethylbenzidine (TMB) substrate was added into each well, followed by the addition of an acid solution. Finally, the change in the enzymatic substrate was determined from double wavelength absorption at 450 nm and between 600 and 630 nm. The absorption measurement was associated with the AMH concentration. AMH calibrator was used for determining the calibration curve of absorption with AMH concentration. The concentration of AMH was calculated from the calibration curve.

2.5. Statistical Data Analysis
The primary output was the FF AMH level. After data collection, verification data editing and coding were performed. All data were analyzed statistically with SPSS 20. The p value was considered statistically significant at 5% with 95% confidence interval. Correlation between the FF AMH levels and the number of oocytes were analyzed using Spearman correlation.

3. Results
Medical records of 20 women with PCOS were collected from the Department of Obstetrics and Gynecology, Yasmin Clinic, Cipto Mangunusomo hospital. All diagnoses were made by doctors who treated patients using Rotterdam criteria.

The subjects were aged 22–39 years (mean age: 32.9 years ± SD 3.18) and had a mean BMI of 25.02 kg/m$^2$ ± SD 3.38. The sample rFSH level had a median value of 2250 (range: 1200–20225).

| Table 1. Sample characteristics |
|---------------------------------|
| Age                             |
| Mean                           | 32.95 |
| Std. deviation                 | 3.18  |
| BMI                            |
| Mean                           | 25.02 |
| Std. deviation                 | 3.38  |
| rFSH                           |
| Median                         | 2250  |
| Range                          | 1200–20225 |
| AMH                            |
| Median                         | 11.59 |
| Range                          | 2.9–24.09 |
The mean FF AMH level in PCOS was 11.59 (range: 2.9–24.09). No significant effect was noted by increase in the AMH levels in the FF on the number of oocytes detected (p = 0.862), with mean AMH levels 5.49 ng/mL showing 18 oocytes. Although the statistical analysis revealed no significance, the correlation graphic demonstrated an increasing trend of AMH levels with decreasing number of oocytes (r = −0.041). (Table 1)

4. Discussion
Increased AMH levels in the FF of PCOS patients can be attributed to the fact that patients with PCOS have increasing number of small antral follicles, which are the major sites of AMH production; this finding is in accordance with that of a previous study by Pellat et al. [6], who demonstrated that AMH production per granulosa cells obtained from anovulatory polycystic ovaries was on an average 75-times greater than that obtained from normal cells. In our study, no correlation was observed between the FF AMH level and the number of oocytes determined during ovum pick-up. However, a different result was reported by Fakher, who found that the serum AMH level can predict the oocyte quantity and quality [7]. The negative result obtained in our study can be attributed to the small sample size, and the AMH level in the FF was not as highly expressed as the AMH level in the serum.

The FF AMH level did not relate to any of the IVF outcomes in patients with PCOS, which is in agreement with the report by Yilmaz [5], who ascertained lack of correlation between FF AMH levels and IVF outcomes in patients who underwent IVF for male infertility or had unexplained infertility. Fanchin et al. [4] reported that the FF AMH concentrations are strongly associated only with the oocyte quality and implantation rates and not with the fertilization rate and embryo morphology under normal ovulation conditions; these findings are in accordance with our study results, which demonstrated a correlation between high AMH levels and decreasing number of oocytes.

Thus, we conclude that the FF AMH level may not be a valuable predictor of the quantity and quality of oocytes in patients with PCOS undergoing IVF. In the future, a study with larger sample size is warranted to confirm our current data.

5. Conclusion
In conclusion there is no correlation between level of AMH in the Follicular Fluid and the numbers of oocytes in the study patients with PCOS.

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