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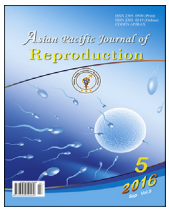

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Serum AMH level predicts oocytes quality better than follicular fluid AMH level

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ABSTRACT

Background: Current *in vitro* fertilization (IVF) methods can't predict oocyte quality after oocyte retrieval. Anti-Mullerian hormones (AMH) in the follicular fluid are produced directly by granulosa cells therefore should be considered as parameter for predicting oocyte quality. The objective of this study is to develop a non-invasive method for predicting oocyte quality.

Methods: This is a prognostic study with a cross sectional design, conducted from April 2012 to June 2013 at Yasmin IVF Clinic – Dr. Cipto Mangunkusumo General Hospital Jakarta. The subjects of this study were infertile couples underwent IVF. The measurement of serum AMH and estradiol level was performed at the beginning of IVF cycles, while follicular fluid AMH, were measured on the day of oocytes retrieval. Oocyte quality was measured using Xia morphology criteria.

Result: We obtained 102 IVF patients with antagonist protocol. Serum and follicular fluid AMH, serum estradiol, number of mature oocytes, and oocytes morphological score were assessed. Serum AMH, follicular fluid AMH and serum estradiol were related to oocytes quality. Based on the multivariate analysis, we found that age and serum AMH level are the best two predictors for oocyte quality.

Conclusion: Serum AMH, but not the follicular AMH, can be used to predict oocytes quality.

1. Introduction

Fifteen percent of the couples of reproductive age experience infertility, and 10% of those cases require *in vitro* fertilization (IVF). Data from several countries throughout the world are included in the 2000 IVF world report, which states that the number of IVF procedures is as much as 191.109 with an average pregnancy rate of 26.7% and an average birth rate of 18.6%. In 2013 in Indonesia, as many as 3 970 IVF cycles with pregnancy rates ranging from 6.15% to 41.5% were reported [1]. The study by Patrizio [2] showed that only 5% of oocytes can produce a baby in an assisted reproductive technology cycle. The factors known affected the success of IVF are the cause of the infertility and patient's age, both of which affected ovarian response to stimulation, number of oocytes, oocyte quality, fertilization rate, and the number and quality of embryos replaced.

Oocyte quality assessment is the most important and the most difficult part of IVF. Morphological parameters have been studied to determine markers of oocyte quality that indicate that an oocyte has good potential for embryonic development. Khalili [3] concluded that oocyte quality plays an important role in the process of fertilization and embryo development in the IVF program.

Because of the oocyte cumulus complex contact with the follicular fluid, a relationship between hormonal content in the follicular fluid and the degree of maturity and the quality of the oocytes is thought to exist [4]. Estradiol is a steroid hormone that has been demonstrated to be positively correlated with the degree of oocyte maturity. However, serum estradiol alone can not predict oocyte number or quality [5].

Anti-Mullerian hormones (AMH) are a glycoprotein compound produced by the granulosa cells of the follicle. The

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Table 1
Modification of Xia morphological criteria.

Criteria	Score		
	0	1	2
First polar body	–	Fragmented	Intact
Perivitelline space	Large	Normal	Normal
Cytoplasmic granulation	Present (spots, vacuoles, refractile body)	Absent	Absent

hormone levels in both the peripheral blood and intra follicular fluid correspond with the rate of follicular maturation. Wiweko *et al.* [6] showed that serum AMH level can predict biological age even earlier than FSH and antral follicle count; biological age is defined here as the ability of ovarian follicles to produce oocytes. Another study by Bushra [7] and Arabzadeh [8] showed that serum AMH level can be used to predict both the quantity and quality of oocytes. Because AMH affects oocyte development during folliculogenesis, and the levels of AMH in the follicular fluid may affect embryo quality and the ability of the oocyte to function.

The purpose of this study was to determine whether serum AMH levels and follicular fluid can be used as predictors of oocyte quality in patients undergoing IVF.

2. Material and methods

This is a prognostic study with a cross-sectional design at Yasmin IVF Clinic Dr. Cipto Mangunkusumo General Hospital was performed from April 2012 to June 2013. All of the procedures in this study were approved by Ethical Research Committee Faculty of Medicine Universitas Indonesia. Written informed consent was obtained from all of the subjects before joining the study. The study population included infertile women 25–42 years old of age who went through serum and follicular fluid AMH level tests as well as oocytes retrieval to examine oocyte quantity and quality as a part of IVF procedures. Patients with no serum AMH data and no mature oocytes retrieved were excluded. Serum AMH levels were measured on any day during the menstrual cycle except on the oocytes retrieval day, and follicular fluid AMH levels were measured on oocytes retrieval day using an AMH Gen II ELISA (Beckman/Coulter, USA). Oocyte morphology was determined from photographs taken on the day of oocytes retrieval using Nikon eclipse Ti-U software and a media home television. We used a modification of Xia's morphological criteria that included 3 components of the first polar body, perivitelline space and cytoplasmic granulation (Table 1). Morphology scores were measured for all oocytes retrieved with a minimum score of 0 and a maximum score of 6. The mean morphology scores for each patient were used for analysis.

All data were analyzed by a Pearson or Spearman correlation test and a linear regression test using SPSS 21 (SPSS Inc, Chicago, IL) and STATA 12 software to determine the correlations of patient age, serum AMH level, follicular fluid AMH level, and serum estradiol with oocyte quality.

Table 2
Subject characteristics (n = 102).

Variables	Value
Age (years)	35.39 ± 4.255
Etiology (n, %)	
Malefactors	22 (21.5)
Endometriosis	20 (19.6)
PCOS	9 (8.8)
DOR	21 (20.6)
Unexplained	30 (29.4)
Serum AMH (ng/mL)	2.10 (0.91–3.34)
Serum estradiol[a] (pg/mL)	1 367 (136.40–2 773)
Follicular fluid AMH (ng/mL)	1.22 (0.71–2.17)
Total oocyte retrieved	9 (5–15)
Mature oocyte	8 (4–12)
Oocyte morphology score	4.76 (4–5.11)

[a] n = 98.

3. Results

In the present study, of 126 patients who were originally included, data for serum AMH were not available for 22 patients and no mature oocyte was found for another 2 patients; thus, 24 patients were excluded. Figure 1 shows details concerning patients who withdrew or who were excluded from analysis.

Before the correlations of age, serum AMH level, follicular fluid AMH level, and serum estradiol with oocyte quality were tested, the normality of the data distribution was confirmed by a Kolmogorov–Smirnov normality test (Table 2). The correlations of patient age, serum AMH level, follicular fluid AMH level,

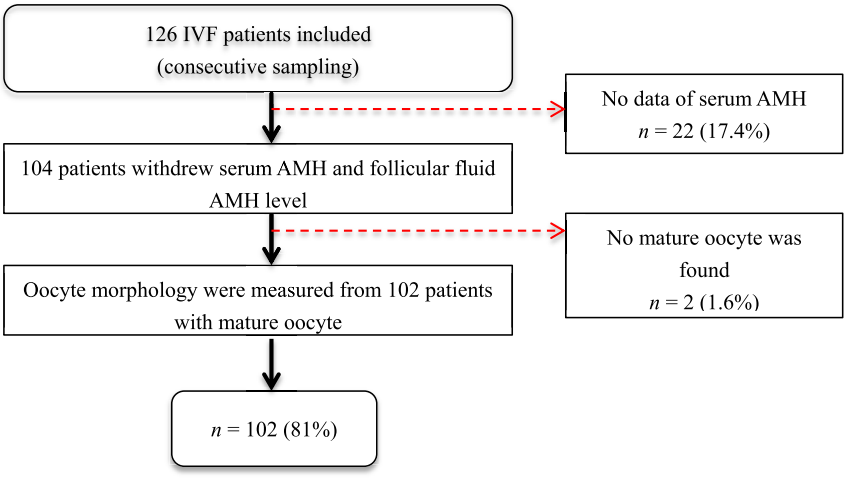

Figure 1. Flow diagram of patients through phases of study.

Table 3
Correlation of variables with oocyte morphology scores (n = 102).

Variables	Morphological score	
	R	*P*
Serum AMH (ng/mL)	0.804	<0.001[a]
Follicular fluid AMH (ng/mL)	0.525	<0.001[a]
Ages (years old)	−0.389	<0.001[a]
Estradiol[b] (pg/mL)	0.278	<0.001[c]

[a] *Pearson* correlation test from transformation data. [b] Using pooled imputation data (n = 98). [c] *Spearman* correlation test.

and serum estradiol with oocyte quality are statistically significant (Table 3).

4. Discussion

Oocyte quality can be assessed in multiple ways, and the methods used have varied among previous studies. However, until now there has been no reasonably objective method for assessing oocyte quality. Guerif *et al.* [9] used oocyte maturity rate (number of oocytes in metaphase II phase divided by the total number of oocytes) as a parameter of oocyte quality, while Rosen [10] used the pregnancy rate parameter. In his review, Revelli [11] found that oocyte quality assessment based on morphology is very popular because it is easy and simple. However, because the method of oocyte selection also varies widely, morphology assessment results tend to be controversial. Rienzi [12] reviewed 50 systematic articles published within the last 15 years on the predictive value of the morphological parameters of mature oocytes. Rienzi found many contradictions in the results of those studies; thus, no conclusions can be drawn regarding oocyte quality parameters based on morphology alone. Until now, there has been no consensus on the standard morphological parameters used for the assessment of oocyte quality.

In this study, we used the first follicular fluid retrieved for examination of AMH, where the first follicular fluid is considered to represent all follicles of the patient. For oocyte quality, we used the average morphology score of all oocytes retrieved. Until now, it was not possible to determine the follicles containing high quality oocytes. As Revelli [11] stated, the results of research on oocyte quality are generally unsatisfactory.

The serum AMH levels were higher than the follicular fluid AMH levels because serum was drawn for AMH testing before stimulation when the follicle diameter is less than 8 mm; thus, AMH in the serum tends to be higher at that time point. Follicular fluid was taken for AMH testing at the time of ovum pick-up, *i.e.* when the diameter of the follicles >18 mm. Andersen [13] showed that AMH levels decrease drastically when follicle diameter >9 mm and then remain low during late follicular development.

Wiweko *et al.* [6] studied chronological age and biological age based on serum AMH levels and found that serum AMH level is an early predictor of ovarian aging and is a more valuable predictor than basal FSH level. A nomogram of the correlation between age and serum AMH level suggested that biological aging occurs at the age of 35 years (50 percentile) and follows a linear pattern. Rosen [10] stated that although the number of antral follicles at a young age has limited predictive value for implantation rate, a decrease in implantation rate at the age of 35 years was obvious, and this decrease became more meaningful over the age of 38 years. The decrease in antral follicle count, which occurs due to aging, can be determined by a decrease in serum AMH level, and serum AMH eventually becomes undetectable in postmenopausal women. Therefore, serum AMH level can be considered a clinical parameter for determining ovarian reserve [14].

The multivariate analysis was performed using the backward method to find the equation for predicting morphology scores. The linear regression analysis results showed that serum AMH level, but not follicular fluid AMH level, can predict oocyte quality. The model of prediction for morphology score uses the following equation: 5 143 − (0.038 × age (year)) + (0.183 × serum AMH (ng/mL)).

Research by Eldar-Geva [15] and Cupisti [16] regarding non-invasive parameters as predictors of IVF success showed that serum AMH level is an important indicator of ovarian function and is the best single predictor of ovarian reserve when compared to other parameters, such as serum inhibin B, estradiol and activin A, in IVF patients with controlled ovarian stimulation. We assume that the follicular fluid AMH level is better at predicting oocyte quality, considering that this fluid is directly in contact with the granulosa cells, which produce AMH. Revelli [11] reviewed the content of follicular fluid to determine the optimal source of biochemical parameters for oocyte quality. Chemical metabolites were identified by that study were categorized into 8 groups as follows: a) hormones; b) superfamily of growth factors of the transforming growth factor-beta (TGF-beta); c) other growth factors and interleukins; d) reactive oxygen species (ROS); e) anti-apoptotic factors; f) proteins, peptides and amino acids; g) sugars; and h) prostanoids. Research on follicular fluid has encountered many obstacles due to the complexity of the biological fluid.

Both serum and follicular fluid AMH level are already known to be positively correlated with oocyte quality. In this study, serum and follicular fluid AMH levels were positively correlated with the total number of oocytes, number of mature oocytes and morphology scores. This is in agreement with the results reported by Lie Fong [17], who found that AMH concentration in the serum was positively correlated with the total number of oocytes. Takahashi [18] showed that follicular fluid AMH level can predict the quality of oocytes: follicular fluid AMH level was higher in oocytes that were successfully fertilized compared to those that were not successfully fertilized. In contrast, the multivariate analysis results from this study indicated that follicular fluid Follicular AMH level cannot be used to predict oocyte quality; instead, oocyte quality was determined with morphology scores (Table 3). This may be due to the sampling method used for the examination of the first punctured follicular fluid AMH level, which is considered to represent all follicles. The morphological score assessment is the average of the morphology scores of all oocytes retrieved from one patient. It is indirectly reflects oocyte quality. Another study by Hattori [19] showed some discrepancy between serum AMH levels and follicular fluid AMH levels in the patients who became pregnant. Some subjects with low serum AMH levels were able to experience good pregnancies. This may be because AMH acts locally on the pre-ovulatory cycles, and in some cases, serum AMH levels do not reflect local AMH levels. In this study, a combination of serum and follicular fluid AMH examination was a strong predictor of pregnancy success.

Serum AMH was found to be significant positively correlated with follicular fluid AMH, total number of oocytes and mature oocytes; the correlation values were moderate to be strong. Serum AMH level can also be used as a predictor of the total number of oocytes and the number of mature oocytes. In conclusion serum AMH predicts the quantity and quality of oocytes better than follicular fluid AMH.

To be concluded, Serum AMH, but not the follicular AMH, can be used to predict oocytes quality. Implication for our daily practice that IVF protocol can be developed based on serum AMH to improve oocytes quality.

Conflict of interest statement

The authors declare that they have no competing interests.

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