

Evaluation of Serum Anti Mullerian Hormone Level in Expected Low Prognosis Group Women Treated of In-Vitro Fertilization/Intra Cytoplasmic Sperm Injection



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Abstract— The use of AMH level in predicting outcomes of in-vitro fertilization/intra cytoplasmic sperm injection in poor responder women has been widely used. Since the term “poor responder” of Bologna criteria has been changed to “low prognosis” according to the POSEIDON (Patient-Oriented Strategies Encompassing Individualized Oocyte Number) criteria, which divided low prognosis into 4 groups, therefore these studies aim to evaluate serum AMH levels in the group suspected of low prognosis women treated with IVF/ICSI. A total of 252 patients of suspected low prognosis groups were assessed retrospectively between 2018 and 2019 in Morula Clinic, National Hospital, Surabaya, Indonesia. Serum AMH cut off values determined for low prognosis groups. Serum AMH levels and pregnancy rate were compared among 4 subgroups of suspected low prognosis women treated IVF/ICSI. In this study, the serum AMH cut off value for predicting low prognosis women using POSEIDON criteria was 1.7 ng/mL with sensitivity 86.7% and specificity 70%. The serum AMH levels were significantly difference in 4 subgroups of suspected low prognosis women treated IVF/ICSI ($p < 0.05$). Interestingly, there were no differences in pregnancy rate among those groups. In conclusion, serum AMH level could predict low prognosis women treated IVF/ICSI. It is important to determine cut-off value to predict the low prognosis women. The pregnancy rate among low prognosis subgroups was not difference.

Keywords— *Anti Mullerian Hormone, low prognosis, IVF, ICSI, maternal health*

Introduction

A success of assisted reproductive technology is influenced by many predictor factors. This factor has been studied well. The major predictors of success are sperm and oocyte quality. Oocyte quality is determined by women age and ovarian reserve. Parameters such as age, antral follicle count (AFC), *Follicle Stimulating Hormone* (FSH), day three cycle of Inhibin and random *Anti-Mullerian Hormone* (AMH) serum during menstrual cycle has been established [1].

AMH is produce during pre-antral and late antral follicle. It is one of the most popular markers to predict outcome ART in terms of pregnancy and cancellation rate in In-Vitro fertilization/ intra cytoplasmic sperm injection (IVF/ICSI) especially for poor responder women. However, the use of AMH as a single marker is still in controversy. Some studies argued about the use of AMH as a single marker, while another study found variation of cut off, sensitivity and specificity [1,2].

In 2011, *European Society of Human Reproduction and Embryology (ESHRE)* defined the women treated of IVF/ICSI as a poor responder and normoresponder. This term is well known as “Bologna Criteria”. These criteria are to help the clinician to predict the outcome and giving the best counseling to the patients. Bologna criteria

Bologna's criteria for determining the status of a women responder treated IVF/ICSI include age, previous ovarian response, and ovarian reserve test [3]. Recently, the POSEIDON (Patient-Oriented Strategies Encompassing Individualize D Oocyte Number) criteria have been established. The POSEIDON criterion

redefines “poor responder” to be a “low prognosis” based on quantitative and qualitative data on age and number of aneuploidy embryos, ovarian markers, history of ovarian response to previous therapy and the ability to take oocytes needed to obtain at least one blastocyst euploidy in each patient[4].

Therefore, this study aims to evaluate the AMH value in women suspected of having a low prognosis for the outcome of IVF/ICSI therapy using POSEIDON criteria.

Methods

This study was a retrospective cohort study. The sample of this study was patients who came to Morula IVF clinic, Surabaya National Hospital Surabaya who met the inclusion criteria.

The criteria for sample inclusion were as follows:

1. Female <45 years
2. An ultrasound was c to determine the AFC
3. AMH examination was performed
4. Willing to sign informed consent

The sample exclusion criteria were as follows:

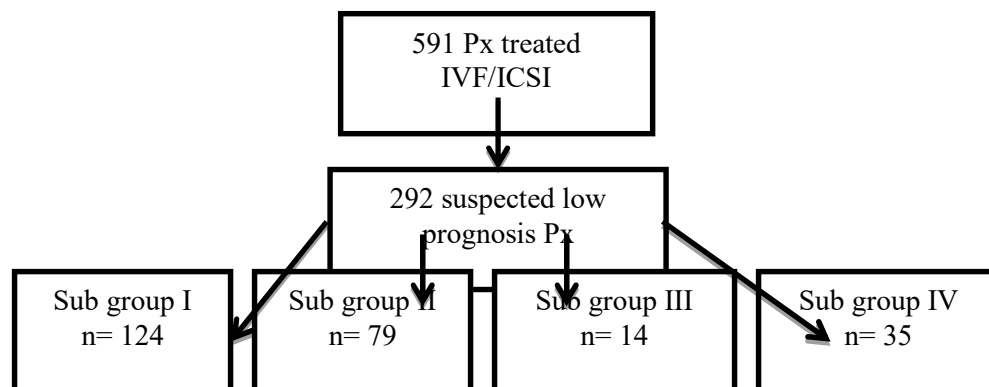
1. PCOS
2. FSH> 20 IU / L

This study was approved by the Ethics Committee of the National Hospital in Surabaya, Indonesia with No 009 / NH / DIR / 2020. Serum AMH levels were analyzed from January 2016-December 2019 data. There were 591 patients who received IVF / CSI therapy. A total of 252 female patients are suspected of having a low prognosis receiving IVF / ICSI therapy. Patients were divided into 4 subgroups with a poor prognosis according to the POSEIDON criteria, namely:

1. Group I: Age <35 years, AFC ≥ 5 , AMH ≥ 1.2 ng / mL, oocyte numbers in previous cycles <9
2. Group II: Age ≥ 35 years, AFC ≥ 5 , AMH ≥ 1.2 ng / mL, total oocyte numbers in the previous cycle
3. Group III: Age <35 years, AFC <5, AMH <1.2 ng / mL
4. Group IV: Age ≥ 35 years, AFC <5, AMH <1.2 ng / mL

Examination of serum AMH levels was performed randomly, using Vidas (Biomeruex ®). The AMH examination method is Enzyme Linked Fluorescent Assay (ELFA).

Patient’s data were obtained from medical records, including Age, infertility factors, number of IVF / ICSI cycles, BMI, AMH, FSH, Estrogen, Progesterone, AFC baseline data with second to fourth day transvaginal ultrasound by one operator. Outcome assessed were post-treatment AFC, oocyte uptake, oocyte quality, pregnancy and number of cycles (Figure 1).



Data were analyzed using SPSS version 21. Kruskal Wallis was used to compare the means in more than 2 groups. Chi-square was used to compare the percentage of pregnancy rate in groups. AUC was used to predict AMH levels in determined the optimal cut-off value. The p value was significant if <0.05

Results

A total of 252 patients were included in this study. Demographic data of patients based on 4 sub groups of low-prognosis were shown in table 1. There were significant differences in the duration of infertility, FSH, oocyte count and post-treatment AFC ($p < 0.001$). However, data of age, BMI, LH, Progesterone were not significantly different (Table 1). Sub groups II and IV had longer infertility than the other sub groups. FSH levels in sub groups II and IV were higher than other sub groups. The number of oocytes and AFC after sub group I and II therapy was higher than the other sub groups.

Table.1 Comparison of parameters for infertility duration, FSH, oocyte count, post-treatment AFC, BMI, LH, P4, E2 in each sub groups

Parameters	Subgroups (mean \pm 2SD)				p value
	I(n=124)	II (n=79)	III (n=14)	IV (n=35)	
BMI (kg/m ²)	37.1 \pm 5.4	39.0 \pm 7.1	40.4 \pm 7.6	36.9 \pm 5.4	0.076
Time of Infertility (year)	4.4 \pm 2.5	8.1 \pm 4.5	5.6 \pm 2.8	8.9 \pm 5.1	0.001
FSH (U/L)	5.4 \pm 1.7	6.6 \pm 2.4	8.1 \pm 3.7	9.8 \pm 3.9	0.001
Oocyte count (n)	14 \pm 7	10 \pm 7	7 \pm 5	4 \pm 4	0.001
AFC post therapy (n)	15 \pm 8	12 \pm 8	7 \pm 7	5 \pm 5	0.001
LH	3.3 \pm 1.3	3.1 \pm 1.4	2.5 \pm 0.8	3.2 \pm 1.3	0.067
P4	0.6 \pm 0.4	0.6 \pm 0.6	0.5 \pm 0.2	0.6 \pm 0.6	0.075
E2	34.4 \pm 14.2	33.7 \pm 16.1	33.9 \pm 15.1	34.5 \pm 15.7	0.659

The optimal cut-off value for determining women with a low prognosis according to the POSEIDON criteria was 1.7 ng / mL with a sensitivity of 86.7% and a specificity of 70% with AUC 0.887 (Fig. 2).

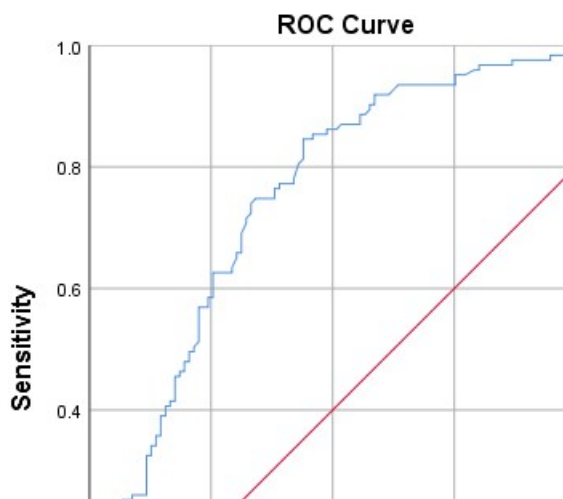


Figure 2. ROC curves of AMH levels in low prognosis.

Interestingly, there were no differences in pregnancy rates in the four sub groups ($p > 0.05$).

We divided the AMH level into 3 sub groups based on the pattern of prior research subgrouping[3] and comparing the parameters of age, duration of infertility, FSH, oocyte count, post-treatment AFC, BMI, LH, P4, E2 in each group (Table 2).

Table.2 Data parameters for infertility duration, FSH, oocyte count, post-treatment AFC, BMI, LH, P4, E2 in each AMH sub groups

Parameters	Group (mean \pm 2SD)			p value
	AMH \leq 0.39 n= 21	AMH 0.4- 2.1 n= 93	AMH >2.1 n= 128	
Age (year)	40.6 \pm 4.96	36.5 \pm 4.91	32.0 \pm 4.43	0.001
BMI (kg/m ²)	36.3 \pm 6.6	38.9 \pm 6.7	37.4 \pm 5.7	0.049
Time of infertility (year)	7.38 \pm 5.48	7.71 \pm 5.14	5.54 \pm 3.77	0.04
FSH (U/L)	10.7 \pm 1.7	7.3 \pm 2.8	5.4 \pm 1.7	0.001
Oocytecount (n)	2.5 \pm 2.0	6.9 \pm 4.0	15.1 \pm 6.8	0.001
AFC post therapy (n)	3.1 \pm 1.5	6.1 \pm 2.9	14.6 \pm 8.3	0.001
LH	3.5 \pm 1.3	2.9 \pm 1.3	3.3 \pm 1.3	0.03
P4	0.4 \pm 0.2	0.6 \pm 0.6	0.5 \pm 0.4	0.520
E2	37.4 \pm 17.3	36.5 \pm 17.9	32.7 \pm 13.6	0.308

Data showed that age, duration of infertility, FSH, oocyte count, post-treatment AFC, BMI and LH were significantly different in each sub group, while P4 and E2 were not significantly different.

DISCUSSION

Since it was introduced in 2011 by the ESHR, "poor responder" patients according to Bologna criteria have been widely studied in terms of the success rate of IVF / ICSI therapy and the failure rate in the form of IVF / ICSI cycle cancellation rates[4, 6-8]. Study showed AMH levels in both serum and follicles have an important role in determining the status of female patients who received IVF / ICSI[9-11]. Many studies showed that it was important to determine the status of female patients who plan to be treated with IVF / ICSI because it could provide the better information for patients about the likelihood of success IVF / ICSI therapy. Another reason was that patient counseling could perform well based on available data including AFC, previous history and AMH levels [3, 9-11].

Recently the term "poor responder" in Bologna criteria was revised to the term "low prognosis" according to the POSEIDON criteria. The changing goal remains the same, namely to provide predictive value to patients, therefore that during the IVF / ICSI therapy process the patient could obtain precise information about the condition and predictions of the success of therapy. POSEIDON criteria added oocyte counts to previous cycles. AMH levels were determined with a cut off of 1.2 ng/mL[5]. However, many study found that 1.2 ng/mL should not be an "absolute" cut off value because AMH levels were vary, depend on ethnic factors and other factors. AMH cut off levels should be determined by the laboratory itself[12-18].

This study found that the optimal AMH level for a woman with a low prognosis was 1.7 ng/mL (AUC 0.876) with sensitivity and specificity of 86.7% and 70%, respectively. This result was in accordance with several other studies which show variations in patients suspected of having a low prognosis. Other studies have found cut-offs to vary from the range 0.5-2.1 ng /mL[7,9,19-20].

Another interesting thing in this study was that we found differences in infertility duration in each sub groups. Groups II and IV naturally become a group with long-standing infertility. This may be caused by age in groups II and IV above 35 years. Previous studies found infertility duration varied in women with a fairly wide range. In post-treatment, the number of oocytes taken was higher in women in sub groups I and II compared to sub groups III and IV. Indicating age did have an effect on oocyte numbers. This was in accordance with previous research which found that the decrease in the number of ovarian reserves and oocyte number occur gradually in accordance with increasing age [3,20].

The hormones estrogen, progesterone and LH in these patients did not differ in each sub group. This gives

an understanding that those hormones have a limited role in ovarian reserve. This found was in accordance to previous studies which concluded that estrogen, progesterone had variations in accordance with the menstrual cycle[3,20].Pregnancy rates in each group were not significantly different ($p > 0.05$). This study found the same result with another study [21].

Determination of the optimal cut-off will help the clinician in determining predictions for the success of IVF / ICSI therapy. We recommend that every laboratory that perform IVF / ICSI therapy determine their own optimal AMH levels on their examinations and continue to evaluate pregnancy rates, number of cycles and cancellation of cycles and births in patients who will undergo IVF / ICSI therapy. Proper counseling will help patients understand the conditions and predictions that are best for their success.

Conclusion

Serum AMH level could predict low prognosis women treated IVF/ICSI. It is important to determine cut-off value to predict the low prognosis women. The pregnancy rate among low prognosis groups was not difference. Further evaluation is needed on the rate of cancellation of cycles and births in each sub group based on AMH predetermined.

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