Research Report

Anti Mullerian Hormone Serum Level Indicates Ovarian Response in Controlled Ovarian Hyperstimulation of IVF Cycles

Kadar Anti Mullerian Hormon sebagai Parameter Respons Ovarium Pasien yang Menjalani Hiperstimulasi Ovarium Terkendali Program FIV

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Abstract

Objective: To evaluate the clinical value of Anti Mullerian Hormone serum (AMH) level as one of ovarian response indicator in controlled ovarian hyperstimulation in IVF cycles.

Method: This cohort-prospective study was conducted in Dr. Cipto Mangunkusumo General Hospital. The subjects of this study were infertile couples who underwent controlled ovarian hyperstimulation in IVF cycles. The measurement of FSH level, estradiol level, AMH level, and antral follicles count was done in the beginning of IVF cycles. The cycles were divided into two groups, good responder group and poor responder group. Good responder group had three or more mature oocytes, while the poor responder group had two or less mature oocytes. Statistical analysis was done using T-Test and Receiver Operator Characteristic area under curve (ROC_{AUC}) to measure the predictive value of AMH, FSH, estradiol, age, and antral follicle count as ovarian response predictors.

Results and Discussion: From 92 IVF cycles, there were 15 poor responder cycles (16.3%) and 77 good responder cycles. AMH serum level was 3.75 ± 2.77 μg/ml in good responder cycles and 1.04 ± 1.39 μg/ml in poor responder cycles ($p < 0.0001$). AMH serum level was more superior than other ovarian response predictors (AUC 0.846) with cut-off value of 1.40 μg/ml. AMH serum level ≥ 1.40 μg/ml had good predictive value as ovarian reserve or ovarian reserve parameter with 81% sensitivity and 87% specificity.

Conclusion: AMH serum level was more superior ovarian reserve and ovarian response predictor compared to other parameters.

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Keywords: anti-Mullerian hormone, ovarian reserve, ovarian response

Abstrak

Tujuan*: Untuk mengevaluasi nilai klinis pengukuran kadar Anti Mullerian Hormone (AMH) sebagai parameter respons ovarium pada program Fertilisasi In Vitro (FIV).*

Metode*: Penelitian dilakukan di RS Dr. Cipto Mangunkusumo dengan rancangan penelitian prospektif kohort. Pasien infertilitas yang menjalani hiperstimulasi ovarium terkendali pada program FIV dilakukan pengukuran kadar FSH, estradiol, AMH dan penghitungan folikel antral basal (FAB). Pasien tersebut dikategorikan menjadi dua kelompok, yaitu perespons baik dan perespons buruk. Kelompok perespons baik memiliki oosit matur ≥ 3 buah saat dilakukan petik oosit. Sedangkan kelompok perespons buruk memiliki oosit matur < 3 buah. Analisis statistik menggunakan uji T tidak berpasangan dan Receiver Operator Characteristic (ROC) untuk melihat apakah AMH lebih superior dibandingkan faktor penduga antara yang lain.*

Hasil dan Pembahasan*: Dari 92 siklus, 15 siklus (16,3%) dikategorikan perespons buruk dan 77 perespons baik. Rerata kadar AMH pada perespons baik adalah 3,75 ± 2,77 μg/ml dan 1,04 ± 1,39 μg/ml pada perespons buruk ($p < 0,0001$). Berdasarkan ROC_{AUC}, AMH merupakan faktor penduga respons ovarium yang baik (AUC 0,846) dibandingkan faktor penduga yang lain seperti usia, FSH, estradiol, dan FAB. Nilai titik potong AMH yang didapatkan adalah 1,40 μg/ml dengan nilai sensitivitas sebesar 81% dan spesifisitas sebesar 87%.*

Kesimpulan*: AMH merupakan faktor penduga respons ovarium yang lebih baik dibandingkan usia, FSH, Estradiol, dan FAB.*

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Kata kunci*: hormon anti-Mullerian, cadangan ovarium, respons ovarium*

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INTRODUCTION

Postponed pregnancy is a challenge for physicians dealing with infertility, because the factors that influence the success treatment of infertility is the age. Physicians are usually faced with the fact that ovarian reserve has decreased with the increasing age of patients. Although the relationship between age and ovarian reserve varies considerably, several cases showed that younger women had lower ovarian reserve than older women, to the extent that objective data were required to determine ovarian reserve.[1,2]

One of the options in the management of infertility is in vitro fertilization (IVF). The relatively high cost of IVF has usually become a concern for infertile couples to participate in the IVF program. Thus, it is necessary to have the examination of ovarian reserve therefore the success of IVF could be predicted.[2]

Several indicators of ovarian reserve include basal follicle stimulating hormone (FSH), basal estradiol, and basal antral follicle (BAF) count.[1] Of these indicators, the number of BAF was the most sensitive.[3] However, the results of the examinations were largely dependent on the type of ultrasonography (USG) used and the skills of its operator, to the extent that a more accurate and easy-to-use examination was needed to determine the exact ovarian reserve. Until recently, doubts have been cast on the accuracy of the value of basal FSH in predicting ovarian reserve because a number of patients with normal FSH values had been found to have inadequate ovarian reserve.

AMH is a dimeric glycoprotein structure and belongs to the group of transforming growth factor β (TGF β). AMH plays a vital role in the development and maturation of follicles. AMH is produced by ovarian granulosa cells since pregnancy age of 36 weeks and impedes the primordial follicle taking during the early taking phase, such that it may prevent depletion of primordial follicles. For this reason, AMH belongs to one of the regulators of folliculogenesis which indirectly describes the number of antral follicles and may provide an indication of ovarian reserve. In addition to being used to identify ovarian reserve, AMH is also beneficial in providing ovarian response. By identifying the level of AMH, the number of mature oocytes obtained could be predicted and the appropriate total amount of gonadotropin dose could be used in the controlled ovarian hyperstimulation in the IVF program.[4,5]

This study aimed to identify the values of AMH, compared to FSH, estradiol and BAF count as the parameters of ovarian reserve and response in IVF. In addition, this study aimed to identify the correlation between AMH level and the total dose of gonadotropin used in the controlled ovarian hyperstimulation in the IVF program.

METHOD

Design of the study was prospective cohort. Eighty four patients who undergoes 92 cycles of IVF at Dr. Cipto Mangunkusumo General Hospital were the subjects of this study. The inclusion criteria were both ovaries could be visualized at USG examination, the number of BAF of the patient could be counted, no history of ovarian surgery, and no history of chemotherapy and radiation.

FHS and estradiol levels were examined on day 2 or day 3 of menstruation cycle, in addition to AMH level measurements on any day throughout the menstrual cycle by using AMH DSL-10-14400 kit. Transvaginal ultrasonography was used to calculate the number of antral follicles on day 2 or day 3 of menstruation cycle using SA Medison 8000 ultrasound.

Ovarian stimulation used both the long-term and short-term protocols. With the long-term protocol, an injection of GnRH agonist was administered at a dose of 500 μg since the intermediate luteal phase for 10 - 14 days. Furthermore, gonadotropin was used at a dose adjusted to the patient's age, the monitoring of estradiol level, and the follicle development at USG examination. Gonadotropin doses used were 150, 225 or 300 IU since the second day of menstruation cycle. GnRH agonist dose was lowered to 250 μg/day. After the first five days of gonadotropin injection, we carried out monitoring follicle growth with ultrasonography and examined blood estradiol level every three days. When at least three follicles with diameter of 18 - 20 mm were found, an injection of human chorionic gonadotropin (hCG) was administered, and oocyte pick-up was performed within 36 hours.

In the short-term protocol, we used GnRH antagonist in which cetrorelix® was administered intravenously with an injection of 0.25 mg every day since day 6 of stimulation or when the largest follicle with a diameter of 14 mm was found. The benefits of using GnRH antagonist include shorter time and smaller number of GnRH analogs employed, and the ability to reduce risks of excessive ovarian stimulation.

In this study, patients were divided into two groups on the basis of the number of oocytes retrieved during oocyte pick-up after the controlled ovarian hyperstimulation. Group with ≥ 3 oocytes is classified into the good responders.

Statistical analysis made use of SPSS 16. The statistical test was carried out using unpaired T test, while Receiver Operator Characteristic (ROC) was used to observe the sensitivity and specificity of each variable.

RESULTS

Eighty four women participated in 92 IVF cycles were included in the current study. These women were subjected to the examination of basal FSH, basal estradiol, AMH levels, and the calculation of BAF. They were divided into two groups based on the ovarian response to the stimulation. If three or more mature oocytes were found, they would be grouped into the cycle of good responders. On the other hand, if two or less mature oocytes were found, they would be grouped into the cycle of poor responders. Fifteen out of 92 cycles (16.3%) were classified as the cycles of poor responders, and 77 cycles (83.7%) were considered the cycles of good responders.

Data of patients' characteristics were shown in Table 1. Of 92 cycles, 18 cycles (20%) were found to experience clinical pregnancy. And 86 cycles (93.5%) constituted primary infertility. Based on the causes of infertility, the most common factors were male factor (44/92; 48%), tubal factor (16/92; 17.4%), endometriosis (8/92; 8%), combined female and male factors (13/92; 14.3%). The protocols used were the long-term protocol (70/92; 76.2%), short-term protocol (14/92; 15%), and the rest was minimal stimulation (8/92; 8.8%). Older patients with diminished ovarian reserve were stimulated using the protocol of minimal stimulation.

Table 1 shows the factors associated with ovarian reserve, i.e. age, FSH level, the number of BAF, and AMH level. It shows that younger patients had better ovarian reserve and response. In addition, lower FSH level correlates with better ovarian response. This was due to the fact that if there was some poor ovarian reserve found in patients with diminished ovarian reserve, the basal FSH level would be higher. It was obvious that patients with better ovarian response and belong to the group of good responders were those with younger ages, lower FSH level, a greater number of BAF, and higher AMH level.

Table 1. Characteristic data of good and poor responders groups.

	Good respond (n = 77)	Poor respond (n = 15)	p value
Age (year)	34.45 ± 4.29	38.33 ± 4.53	0.001
IMT (kg/m²)	23.14 ± 3.18	23.68 ± 3.89	0.639
FSH level (IU/l)	7.06 ± 3.40	13.64 ± 8.43	0.010
Estradiol level (pmol/l)	50.7 ± 38.5	51.1 ± 39.63	0.976
AMH level (g/ml)	3.75 ± 2.77	1.05 ± 1.39	0.0001
BAF (number)	8.88 ± 3.4	4.47 ± 2.88	0.0001
Mature oocyte (number)	7.28 ± 3.33	1.55 ± 0.52	0.0001

Diagram of ROC_{AUC} of the four variables that indicate ovarian reserve can be seen in Figure 1. Cut-off point for age is 35 years in which age less than 35.5 years had a good prediction of ovarian response, with sensitivity of 64% and specificity of 57% (AUC = 0.707; p = 0.012; 95% CI 0.585 - 0.829). FSH level had a cut-off point of 7.25 IU/l in which FSH level lower than 7.25 IU/l had a good prediction of ovarian response, with sensitivity of 73% and spe-cificity of 74% (AUC = 0.765; p = 0.002; 95% CI 0.619 - 0.910). The number of BAF had a cut-off point of 7.5 in which the number of BAF greater than seven follicles had a good prediction of ovarian res-ponse, with sensitivity of 73% and specificity of 82% (AUC = 0.843; p < 0.0001; 95% CI 0.732 - 0.959). AMH level had a cut-off point of 1.40 µg/ml in which AMH level higher than 1.40 µg/ml had a good predic- tion of ovarian response, with sensitivity of 81% and specificity of 87% (AUC = 0.846; p < 0.0001; 95% CI 0.735 - 0.968). These findings are shown in Figure 1.

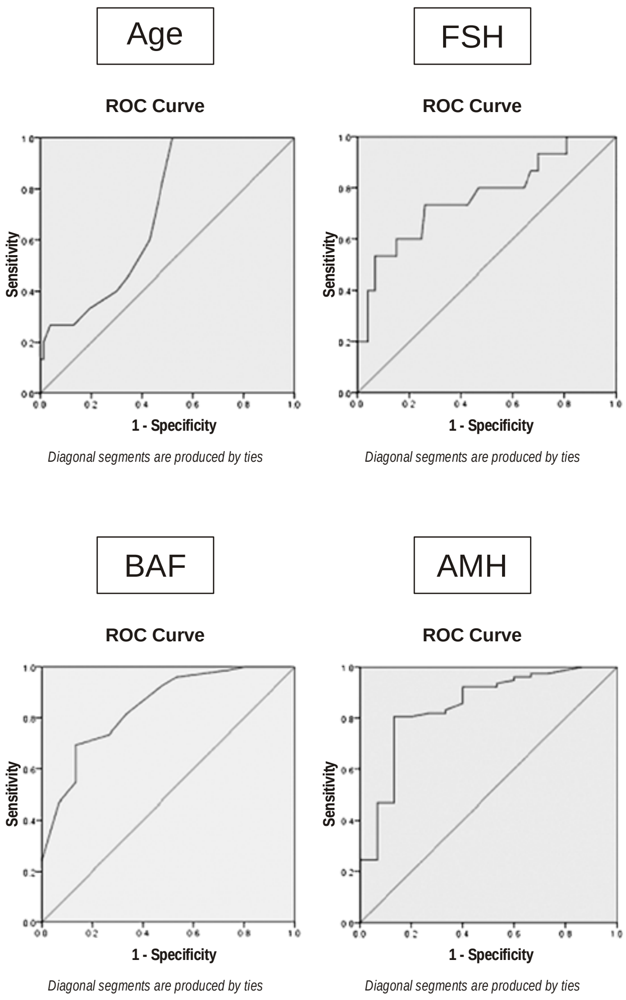

Figure 1. Receiver Operator Characteristic (ROC).

Table 2. ROC_{AUC} Age, FSH level, BAF, and AMH level in predicting ovarian response.

Predictive factor	ROC_{AUC}	Significance
Age	0.707	0.012
FSH	0.765	0.001
BAF	0.843	< 0.0001
AMH	0.846	< 0.0001

Multivariate analysis of factors affecting the ovarian response showed that the most dominant factor was AMH, followed by the number of BAF. On the other hand, age and FSH level were not included in the analysis of linear regression since they did not meet the requirements (p > 0.25). Hence, we compose a linear regression formula for ovarian reserve and response, where mature oocyte counts = 1.544 + 0.686 x AMH level + 0.356 x BAF counts. If only one variable was present, i.e. AMH level, the equation of linear regression would be the number of mature oocytes = 3.690 + 0.910 x AMH level. If it was to be based on the number of BAF, the equation of linear regression would be the number of mature oocytes 1.745 + 0.607 x the number of BAF. This could be seen in Figure 2. Therefore, it can be concluded that AMH level was the most dominant factor in determining the ovarian reserve and response, which was followed by the number of BAF.

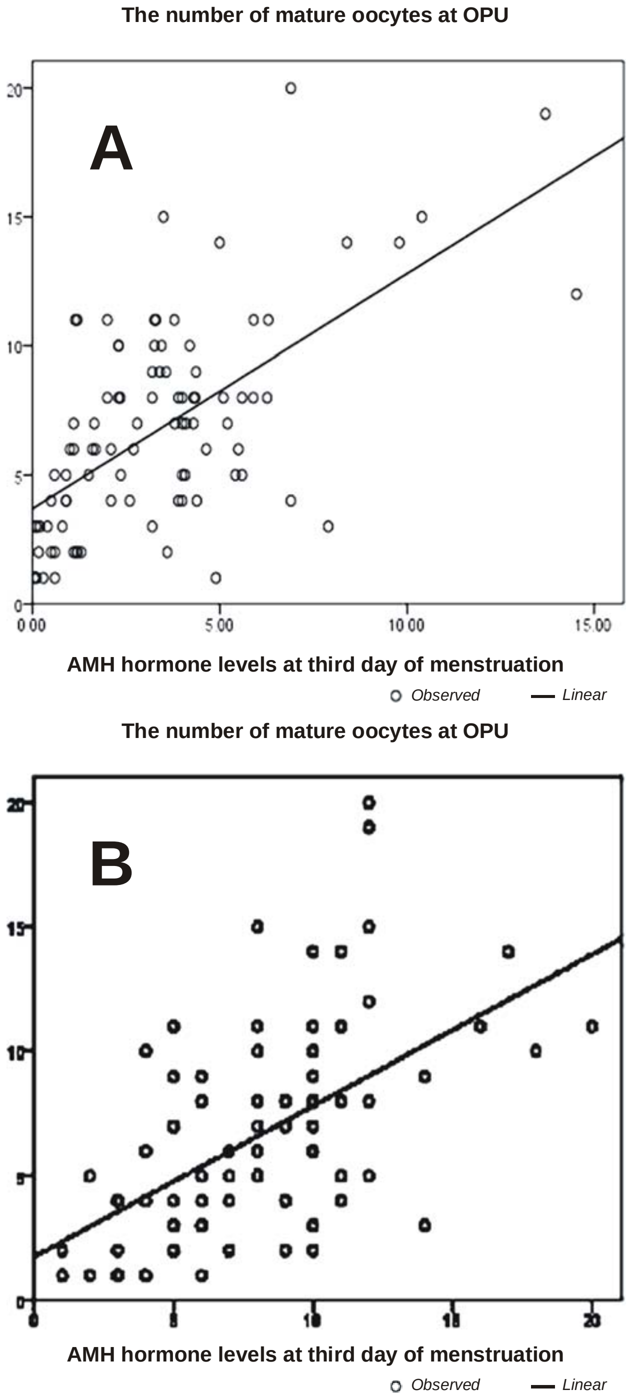

Figure 2. The equation of linear regression, i.e. the number of mature oocyte = 3.690 + 0.625 x AMH level (**A**) and the number of mature oocytes = 1.745 + 0.549 x the number of BAF (**B**).

Figure 2 shows the equation of linear regression, i.e. the number of mature oocyte = 3.690 + 0.625 x AMH level (above) and the number of mature oocytes = 1.745 + 0.549 x the number of BAF (below). If both variables were combined, they would result in the following equation of linear regression: the number of mature oocytes = 1.544 + 0.468 x AMH level + 0.322 x the number of BAF.

In addition to serve as a useful predictive factor of the number of mature oocytes, AMH level had an inverse correlation to the total dose of gonadotropin

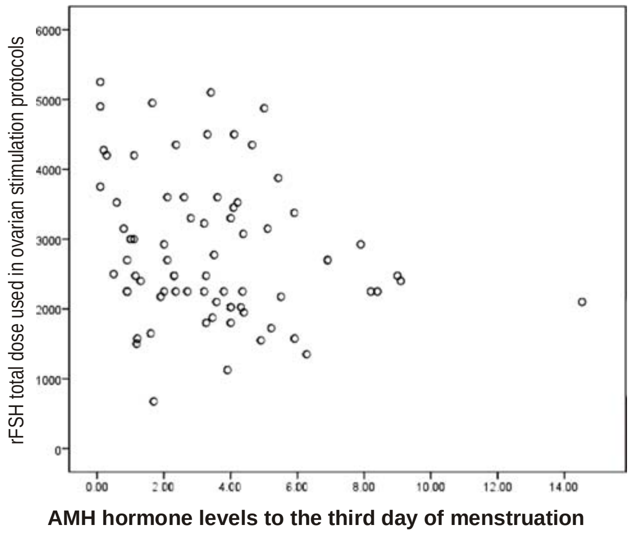

Figure 3. Correlation between total doses of gonadotropin used in IVF and AMH level.

used in IVF ($p = 0.047$; $r = -0.232$). This could only be applied in the long-term protocol or short-term protocol, not in the minimal stimulation which was usually applied in patients with diminished ovarian reserve. In the current study, if the natural cycle was excluded then we would have an inverse correlation between AMH level and the total dose of gonadotropin. If AMH level in the early period of IVF was found to be high, the dose of gonadotropin used should be lower than if AMH level in the early period of IVF was low, in order to achieve the similar ovarian response. These findings could be seen in Figure 3.

DISCUSSION

In this study, the cycles with good ovarian reserve and response were found in patients with younger age. The correlation between ovarian response and age was indubitably clear. All indicators of ovarian reserve correlated with age. Although in several different cases a decrease in ovarian reserve was found, in women with the same ages.[1,6,7] The causes of this condition include the use of chemotherapy, radiation, history of ovarian surgery, and others.[8,10] On the other hand, body mass index (BMI) did not show any significant difference between both groups ($p = 0.639$) in this study.

The AUC value of AMH level (AUC 0.846) was the highest in comparison with other factors, followed by the number of BAF (AUC 0.843). This shows that AMH level constituted the most superior parameter in this study, a finding that was consistent with the study conducted by Visser et al[11] and Kwee et al.[12] AMH level is the most novel and superior predictive factor of ovarian reserve and response. AMH level had several benefits compared with BAF count. The results of BAF count were dependent on the type of USG used and the skills of its operator, while with AMH only venous serum was needed for its examinations. Furthermore, AMH was not affected by menstrual cycle, something different from FSH and estradiol levels which should be examined on day two or day three of the menstrual cycle.

In addition, to identify ovarian reserve, AMH level could also be employed to predict the total dose of gonadotropin used in controlled ovarian hyperstimulation in IVF program. This was due to the fact that there was a correlation between AMH level and the total dose of gonadotropin used in this study. This finding was consistent with the study performed by Kwee et al.[12]

CONCLUSION

Anti Mullerian Hormone (AMH) has the benefits in predicting ovarian response toward ovarian stimulation in the IVF program. Overall, AMH was more superior than other predictive factors. AMH level also has a correlation with the total dose of gonadotropin used in controlled ovarian hyperstimulation in IVF.

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