Comparison of Anti-Mullerian Hormone (AMH), Basal Follicle Stimulating Hormone (FSH), Estradiol and Antral Follicles Count as Predictors of Ovarian Response in in vitro Fertilization Program

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ABSTRACT

*Ovarian stimulation response is crucial to be identified in in vitro fertilization program. There are several markers to identify such response, including AMH, basal FSH, estradiol and AFC. This study aimed to find the difference AMH, basal FSH, estradiol and AFC as ovarian responsepredictors. This was an observational analytic study in infertile patients. Subjects performed blood sampling 3-day menstrual cycle for inspection of AMH, basal FSH, E2 basal and antral follicle number calculation (AFC). Based on oocytes the number, the subjects were divided into groups of poor responders (≤ 4 oocytes) and good responders (oocytes 5-15). We performed correlation and regression test between AMH, basal FSH, AFC, and basal E2 with three indicators of ovarian stimulation response (number of oocytes at OPU, the number of preovulatory follicles and E2 levels at hCG administration).The results showed a significant difference in AMH levels (p = 0.001), basal FSH (p = 0.045), and AFC (p = 0.001) between poor and good responders. AMH correlated strongly and significantly with OPU (r = 0843, p = 0.001), number of preovulatory follicles (r = 0577, p = 0.001), and current E2 administration HCG (r = 0701 and p = 0.001). AFC also show a strong and significant correlation with the OPU (r = 0721, p = 0.001), number of preovulatory follicles (r = 0555, p = 0.002), and current E2 administration HCG (r = 0435, p = 0021). Only basal FSH obtained by OPU had significant correlation (r = -0420, p = 0026). Basal E2 showed no significant correlations on all three indicators of ovarian response. In conclusion, AMH is a better predictor of ovarian response than AFC, basal FSH and basalE2.(**MOG 2013;21:84-88**)*

Keywords: *AMH, basal FSH, basal E2, AFC, ovarian response, in vitro fertilization*

ABSTRAK

*Respons stimulasi ovarium penting untuk diketahui pada program vertilisasi in vitro. Terdapat beberapa marker untuk mengidentifikasi respons tersebut, meliputi AMH, basal FSH, estradiol dan AFC. Penelitian ini bertujuan mengetahui perbedaan AMH, FSH basal, estradiol dan AFC sebagai prediktor respon ovarium. Penelitian ini adalah penelitian analitik observasional pada pasien infertil. Sampel darah 3 hari siklus menstruasi diambil untuk pemeriksaan AMH, basal FSH, jumlah folikel E2 basal dan antral (AFC). Subyek dibagi menjadi kelompok responder buruk (≤ 4 oosit) dan baik (oosit 5-15). Kemudian dilakukan uji korelasi dan regresi antara AMH, basal FSH, AFC, dan E2 basal dengan tiga indikator respon stimulasi ovarium (jumlah oosit pada OPU, jumlah folikel preovulasi dan kadar E2 saat pemberian hCG). Analisis menunjukkan perbedaan signifikan pada tingkat AMH (p = 0,001), basal FSH (p = 0,045), dan AFC (p = 0,001) antara kelompok responder buruk dengan responder baik. AMH memiliki korelasi yang sangat kuat dan signifikan (r = 0843, p = 0,001) dengan OPU, jumlah folikel preovulasi (r = 0577, p = 0,001) dan E2 pada pemberian hCG (r = 0701, p = 0,001). AFC juga menunjukkan korelasi kuat dan signifikan dengan OPU (r = 0.721 dan p = 0,001), jumlah folikel preovulasi (r = 0555 dan p = 0,002), dan hCG pada pemberian E2 (r = 0.435 dan p = 0021). Di sisi lain, hanya FSH basal diperoleh OPU yang memiliki korelasi signifikan (r = -0.420 dan p = 0026). Simpulan: AMH adalah prediktor yang lebih baik untuk respon ovarium daripada AFC, FSH basal dan E2 basal.(**MOG 2013;21:84-88**)*

Kata kunci: *AMH, FSH basal , E2 basal, AFC, respon ovarium, fertilisasi in vitro*

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INTRODUCTION

Age is one of the main factors affecting the reproductive system. It has been known for years that a woman's ability to have children (female fecundity) declines with increasing age.[1] Women aged over 30 years compared with younger women in the normal population showed a decrease of 33% to have a child.[2] One of the factors that play a role in the pathophysiology of decreased fecundity due to increased age is a decline in ovarian reserve (ovarian reserve). Decline in ovarian reserve or the largest

follicles occur at 16 weeks' gestation in which 6-7 million follicles down to around 2 million at birth. Then drop the follicle reserve is running relatively stable around 1000 follicles each month and at the age of 35 years only about 100 follicles up to 1000 follicular alone.[3] So with the invitro fertilization is one solution for infertile couples desiring offspring.[4]

Other markers of ovarian reserve are currently widely studied is Anti-Mullerian hormone (AMH).[5] Mullerian inhibiting substance (MIS) or AMH is a dimer glikoproterin included in the transforming growth factor-beta (TGF-β) superfamily that is one hormone that can be used as a marker of ovarian function.[6] AMH was first recognized in 1947 as a factor affecting the Mullerian duct regression in male fetuses. Since that time the function of the widespread use of AMH as a marker of neonatal Sertoli cells, the determinant of the identification and development of intersex disorders recently used as a marker in folikulogenesis.[7] AMH in women mainly produced by the granulosa cells of preantral follicles with a diameter of 2-6 mm, which had a role in the development of follicles.[8] AMH is secreted by the ovaries into the circulation so that AMH levels can be measured in serum. AMH levels in serum may reflect the ovarian follicular reserve so that when a decline in the number of follicles will be followed by a decrease in circulating AMH.[9]

Until now in infertility or fertility clinic at Dr. Soetomo Hospital is still using the FSH levels on day 3 of the menstrual cycle to assess the ovarian reserves. This condition causes one patient could not be assessed at any time. These fact is contributed to the low number of patient visits to a fertility clinic because they had to adjust the time due to visit on the 3rd day of the menstrual cycle. Examination of AMH has more value than the other markers that AMH levels are not affected by the menstrual cycle. Thus, AMH is independent of the menstrual cycle as a measure to assess the response of ovarian stimulation.[10] Therefore, this study was conducted in order to determine the differences Anti-Mullerian Hormone (AMH), Follicle Stimulating Hormone (FSH) basal, basal antral follicle count and the number of basal estradiol as a predictor of response to ovarian stimulation in in vitro fertilization program

MATERIALS AND METHODS

This study was observational analytical prospective cohort study design in humans by observing and measuring several variables on study subjects infertile women who mengkuti IVF. The study was conducted in Graha Amerta Fertility Clinic and Laboratory of Clinical Pathology Dr. Sutomo at Hospital Surabaya. The research sample taken from the population, ie patients who come to RSU Dr Sutomo Surabaya is infertile patients undergoing IVF in RSU Dr Sutomo Surabaya, women of reproductive age and menopause and given their consent to follow this study.

All patients did AMH levels check without notice day menstrual cycle and then do the recording age, weight and height measurements. Then examined to assess the oocyte reserve made on the 3rd day of the menstrual cycle and vaginal ultrasound to count the follicles antral. Then performed ovarian stimulation initiated by administering exogenous gonadotropins in the form of recombinant FSH (Gonal-F, Serono) from the 3rd day of the menstrual cycle at starting dose adjusted by age and basal FSH levels. GnRH antagonist given once on day 8^{th} of the menstrual cycle at a dose of 3 mg subcutaneous injection given to prevent premature LH surge. Ovarian stimulation response assessed 36 hours after hCG injection is by counting the number of oocytes that can be retrieved at OPU. Then the subjects were classified into three groups: (i) the poor responder, when oocytes were obtained $\leq$ 4 oocytes, (ii) normal responder group, when oocytes were obtained between 5-16 oocytes (iii) the high responder group when oocytes were obtained > 16 oocytes.

The data obtained by multiple linear regression analysis to look at the relationship and know the comparison between the variables under study and to determine the independent variables are more influential in response to ovarian stimulation for in vitro fertilization program participants. The entire statistical tests used in this study using the limit of significance $p < 0.05$.

RESULTS

The research was carried out for 5 months and gained 33 subjects who participated in the study, but four (12.1%) subjects did not continue the study because of moving out of the city of residence and for no reason (does not come as scheduled control). Furthermore, the cause of infertility of 29 remaining subjects is 10 subjects or 34.5% due to tubal factor, 6 subjects or 20.7% due to male factor, 5 subjects or 17.2% due to ovulation disorders, 5 subjects or 17.2% due to factors unexplain, and 3 subjects or 19.3% because of the endometrium. While the characteristics of variables is shown in table 1. Average AMH levels in poor responders are 1:38 ± 0:18 ng/ml with a value of (1.06 ng/ml – 1.58 ng/ml). While the average levels of AMH in good responder group subjects was 4:06 ± 0.82

ng/ml (2.1 ng/ml - 4.95 ng/ml) and this is a significant difference between the two groups (*p = 0.001*).

To compare independent variables as predictors of response to ovarian stimulation in this study using multiple linear regression test (Table 2) is to test each independent variable (AMH, basal FSH, AFC, basal and E2) with each of the dependent variable (number of oocytes at OPU, the number of preovulation follicles and E2 levels at hCG administration).

DISCUSSION

Ovarian response in this study is assessed based on the number of oocytes obtained at OPU, the number of preovulatory follicles and E2 levels at hCG administration. Of the three parameters of ovarian response, AMH has a very strong correlation and significant in all three of these variables (AMH correlation with OPU obtained r = 0843 and p = 0.001, AMH correlation with the number of preovulation follicles obtained r = 0577 and p = 0.001, and the correlation of AMH with E2 obtained r = 0701 and p = 0.001). This suggests that AMH is a reliable predictor of ovarian response.

Research that has been largely recommended AMH used as predictors of response to ovarian response IVF.[9,11,12,13] AFC in this study also had a strong correlation to the three indicators of response to ovarian stimulation (AFC correlations with OPU obtained r = 0721 and p = 0.001, correlation with the number of preovulation follicles obtained r = 0555 and p = 0.002, and correlation with E2 obtain r = 0435 and p = 0021).

Multiple linear regression analysis used in this study to find which variables of the four variables studied as a predictor of the best in assessing ovarian response. Of the three indicators of ovarian response, AMH and AFC affect the number of oocytes at OPU but regression equations derived from AMH has a value greater influence (1.422) is almost three times the AFC (0.365). In ovarian response indicator of preovulation follicle only AMH is included in the multiple linear regression equation. This means that in this study only AMH that significantly affect the number of preovulation follicles. This condition can occur because the AFC accuracy depends on the tools and expertise of sonographers that bias affecting the measurement results is quite large. The opposite, in AMH measurement is not needed because it takes a certain skill expertise to take venous blood only.

A study showed that follicle antral diameter 2-6 mm has a strong correlation with the condition of ovarian reserve and ovarian response in controlled ovarian stimulation (COS) compared to follicles with a larger diameter.[14] AFC measurements performed on follicles with a diameter of 2-10 mm in contrast to the specific AMH is produced only by antral follicles with a diameter of 2-6 mm (some literature there is mention of 2-7 mm), this condition causes AMH has a better correlation to the stimulation response Ovarian because AMH may reflect ovarian reserve better than the AFC.

While on the other ovarian stimulation response indicators, namely the current E2 administration HCG levels AMH does not fit in multiple linear regression equation, but this is not significant because current E2 indicator of hCG administration was not shown to be significantly correlated with four independent variables (AMH, AFC, basal FSH and E2).

Table1. Characteristics of variables based on the response of ovarian stimulation

Variable	Poor Responder		Good Responder		*p*
	Mean	Range	Mean	Range	
Age	32.33 ± 3.54	27 – 38	32.83 ± 4.59	25 - 40	0.051
BMI	27.07 ± 2.17	23.12 – 29.65	26.75 ± 2.10	21.12 – 29.55	0.117
Basal FSH	10.60 ± 6.43	5.93 – 25.53	6.53 ± 2.06	0.66 – 9.69	0.045
Basal E_2	27.32 ± 15.81	8 – 62	33.44 ± 14.25	12.23 – 72.85	0.377
AMH	1.38 ± 0.18	1.06 – 1.58	4.06 ± 0.82	2.1 – 4.95	0.001
AFC	2.22 ± 0.66	1 – 3	7.11 ± 1.81	3 – 9	0.001

Table2. Multiple linear regression results of AMH, basal FSH, AFC, and E2 withovarian response indicator

Ovarian response indicator	linear regression (*y*)	*p*	Description
number of oocytes at OPU	- 1.01 + 1.422 AMH + 0.365 AFC	0.001 and 0.034	FSH and Basal E2 were removed (p > 0.05)
preovulation follicles	3.005 + 1.608 AMH	0.001	FSH,AFC,Basal E2 were removed(p>0.05)
E2 levels at hCG administration	577.98 + 140.53 AFC	0.002	FSH,AMH, Basal E2 were removed(p>0.05)

The results of this study suggest AMH and AFC have more intense correlation with indicators of ovarian stimulation response than other hormonal markers (basal FSH and E2). The reason for this phenomenon is certainly not known with certainty, but may be related to the different regulations of each of these hormonal markers. During the luteal-follicular transition, E2 secretion by modulating early antral follicles themselves with FSH stimulation.

This means that the levels of E2 not only dependent on the most active granulosa cells available, which is represented by the number and size of follicles, but also influenced by FSH stimulation. Different things happen at AMH, AMH expression during the early follicular phase is not affected by FSH or in other words less FSH hormone-sensitive compared with E2. Therefore, AMH is a marker of a more independent and reliable than basal FSH and E2.

CONCLUSION

The results of this study showed that serum AMH has a very strong positive correlation and significant in all three indicators of ovarian response, and the number of oocytes are generated when picking ovum, preovulasi number of follicles and estradiol levels of hCG administration and AMH as a predictor of response to ovarian stimulation better than the AFC, basal FSH and E2 basal

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