Research Report

Effect of Ovarian Stimulation with Recombinant FSH for In Vitro Fertilization (IVF) on Anti Müllerian Hormone (AMH) Levels as an Early Marker of Ovarian Reserve

Pengaruh Stimulasi Ovarium FSH rekombinan dengan Fertilisasi In Vitro (IVF) pada tingkat Hormon Anti Müllerian (HAM) sebagai Penanda Dini Cadangan Ovarium

Kartika Hapsari[1], Budi Wiweko[1], Joedo Prihartono[2]

[1]Department of Obstetrics and Gynecology
[2]Department of Community Medicine
Medical Faculty of Indonesia University/
Dr. Cipto Mangunkusumo Hospital
Jakarta

Abstract

Objective: To analyze the effect of ovarian stimulation with recombinant FSH on AMH levels as a marker of ovarian reserve in patients undergoing IVF.

Method: This study is an intervention study which compares AMH levels pre- and post-ovarian stimulation with recombinant FSH in IVF participants to determine the effect of ovarian stimulation on ovarian reserve. This study was conducted at Dr. Cipto Mangunkusumo Hospital, Jakarta, from January to July 2010. AMH levels measured are AMH levels pre- and post-stimulation taken on the day of hCG evaluation.

Result: Of 56 patients undergoing IVF treatment, 20 subjects were eligible for study. The mean age of the patients was 35. 3 ± 4.0 years, the mean duration of infertility 9.1 ± 5.7 years, the mean BMI 21.9 ± 3.4 kg/m^2, the median AMH level pre-stimulation was 4.0 ng/ml and the total dosage of FSH used was 2747.5 ± 1076.3 IU/day, the mean duration of ovarian stimulation was 9 ± 3 days. Of the 20 subjects recruited, 11 patients (55%) had a decrease in AMH levels, and the remaining 9 patients (45%) did not. Data analysis showed that the decrease in AMH levels was not statistically significant (p = 0.295) [AMH_1; median 4.0 ng/ml (range 2.2 - 5.9) and AMH_2; median 3.2 ng/ml (range 1.6 - 4.8)].

Conclusion: AMH levels decrease following ovarian stimulation with recombinant FSH is not statistically significant, so it can be concluded that ovarian stimulation in IVF has no effect on ovarian reserve.

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Keywords: ovarian reserve, AMH, recombinant FSH, ovarian stimulation, IVF

Abstrak

Tujuan*: Menganalisis pengaruh perangsangan ovarium dengan FSH-rekombinan terhadap kadar AMH sebagai tolak ukur cadangan ovarium pada pasien peserta FIV.*

Metode*: Penelitian ini merupakan penelitian intervensi yang bertujuan untuk membandingkan AMH pra- dan pasca-perangsangan ovarium dengan FSH rekombinan pada peserta FIV untuk menilai pengaruh perangsangan ovarium terhadap perubahan cadangan ovarium. Penelitian dilakukan di Rumah Sakit Dr. Cipto Mangunkusumo, Jakarta. Penelitian berlangsung bulan Januari hingga Juli 2010. Kadar AMH yang dinilai adalah kadar AMH pra- perangsangan ovarium dan pasca-perangsangan yang diambil pada hari pemeriksaan hCG.*

Hasil*: Dari 56 pasien baru peserta program FIV, didapatkan 20 subjek yang memenuhi kriteria inklusi. Rerata umur pasien adalah 35,3 ± 4,0 tahun, lama infertilitas pasien 9,1 ± 5,7 tahun, IMT 21,9 ± 3,4 kg/m^2, nilai tengah kadar AMH awal 4,0 ng/ml dan total dosis FSH yang dipakai adalah 2747,5 ± 1076,3 IU/hari, rerata periode perangsangan ovarium dilakukan selama 9 ± 3 hari. Dari 20 subjek penelitian didapatkan 11 pasien (55%) mengalami penurunan AMH dan sisanya 9 pasien (45%) yang tidak mengalami penurunan. Dari analisis didapatkan penurunan kadar AMH yang tidak bermakna (p = 0,295) dengan nilai median AMH_1 4,0 ng/ml (rentang 2,2 - 5,9) dan median AMH_2 3,2 ng/ml (rentang 1,6 - 4,8).*

Kesimpulan*: Kadar AMH menurun secara tidak bermakna setelah proses perangsangan ovarium dengan FSH rekombinan, sehingga dapat dikatakan bahwa proses perangsangan ovarium pada FIV tidak berpengaruh langsung pada cadangan ovarium.*

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Kata kunci*: cadangan ovarium, AMH, FSH rekombinan, perangsangan ovarium, IVF*

Correspondence: Kartika Hapsari, Department of Obstetrics and Gynecology, Medical Faculty of Indonesia University/ Dr. Cipto Mangunkusumo Hospital, Jakarta. Telephone: 021-70699987. email: tika_tedjo@yahoo.com

INTRODUCTION

Fertility problems in Indonesia is increasing. According to a report from the Department of Health, Indonesia, in 2010, approximately 12% of couples of child-bearing age have fertility issues. Causes of infertility are classified into three groups; 40% of the causes are attributed to women, 40% attributed to men, and 30% idiopathic (unexplained, unknown causes).[1] Management of infertility depends on the main cause. Problems arise when a variety of methods to achieve pregnancy had been done to no avail.

There are currently various methods of assisted reproductive technology (ART), both available and under development, which can solve the problem of infertility for couples of child-bearing age who had previously tried various ways to achieve pregnancy with no satisfactory results. Of assisted reproduction techniques, IVF is the most commonly used.[2] In this method, recombinant FSH injection was given to stimulate follicle development, a process known as controlled ovarian stimulation.[2] This process is a crucial part of the IVF treatment, as ovarian stimulation will produce more oocytes, therefore, increasing the number of embryo available for tranfer.[2] Various hormonal medications and methods used to stimulate the ovaries have been developed over time. One of the earliest method used was the combination of clomiphene citrate and human menopausal gonadotropin (hMG) derived from the urine of postmenopausal women. Followed by synthetic gonadotropin-releasing hormone (GnRH) analogs and recombinant FSH.

In the last 2 years, there were a significant number of women who underwent ovarian stimulation through IVF programs as well as through artificial inseminations, moreover, in the world, there is a definite upward trend of this number. In developed countries, approximately 56.1 - 80% of infertile couples seek treatment for infertility, and 17 - 30% will undergo IVF program.[3]

The increase in this number is accompanied with concern about the occurrence of premature menopause as a side effect of ovarian stimulation. This theory is based on the possibility of accelerated antral follicle uptake due to the recombinant FSH given. Pines and colleagues, in 2002, reported that 55 - 60% of women who underwent ovarian stimulation will experience menopause 5 years earlier than those who did not.[4]

This finding was further refuted by studies which reported that ovarian reserve does not undergo significant decrease post-controlled ovarian stimulation. Among these studies were Chan et al., 2005, who performed ovarian reserve test (ORT) post stimulation by measuring FSH levels[5]; and Frattarelli et al., 2000, who measured the number of antral follicles.[6] These studies stated that ovarian reserve does not undergo significant decrease after stimulation. This can be confirmed by evaluating levels of biomarkers of ovarian reserve pre- and post-ovarian stimulation. Established biomarkers of ovarian reserve used were follicle stimulating hormone (FSH), basal estradiol (E_2) and basal antral follicle (FAB). The most novel method currently used is by determining Anti Müllerian Hormone (AMH) levels. AMH has been reported to be a strong predictor of ovarian reserve with a sensitivity and specificity of 90.9% compared to FSH, E_2, and FAB combined.[7] This can be accounted to its characteristics; AMH levels does not change significantly throughout the menstrual cycle, has a direct correlation with age, produced by pre-antral and antral follicles, directly inhibit primordial follicle depletion and FSH-stimulated growth on follicles. Owing to these characteristics, it can be said that AMH reflects the number of follicles more accurately, it also functions to regulate folliculogenesis and oocyte maturation.[7,8] Previous study done by Wiweko and colleagues stated that changes in serum AMH levels occur earlier than FAB and serum FSH changes, making it a better predictor to immediately evaluate ovarian reserve.[9]

METHODS

This study is an intervention study which aims participants to determine the effect of ovarian stimulation on ovarian reserve by comparing AMH levels pre- and post-ovarian stimulation with recombinant FSH in IVF. Study population is all patients who participated in IVF program and underwent ovarian stimulation process at Dr. Cipto Mangunkusumo Hospital, Jakarta, between January 2010 and July 2010.

Following inclusion criteria were used; women underwent IVF program, normal ovaries, regular 28 - 30 days menstrual cycle, gave consent to participate in the study, did not smoke. Exclusion criteria used were; history of ovarian procedure, ovarian stimulation, radiotherapy and or chemotherapy.

On the second day of the menstrual cycle, 5 cc of venous blood was taken from each patient and stored at -80°C. After 30 minutes, blood samples were centrifuged at 3500 rpm for 15 minutes. Subsequently, the levels of serum E_2, FSH dan AMH were measured at Makmal Terpadu Imunoendokrinologi (MTIE) Fakultas Kedokteran Universitas Indonesia. Determination of serum E_2 (pg/ml) was done with AxSYM Estradiol Microparticle Enzyme Immunoassay (MEIA, Abbott). Determination of serum FSH (m IU/ml) was done with AxSYM Estradiol Microparticle Enzyme Immunoassay (MEIA, Abbott). Determination of serum AMH (ng/ml) was done with enzyme-immunometric (ELISA, DSL, Abbott).

On the second day of the menstrual cycle, transvaginal USG was done to each patient using ALOKA SSD 3500 ultrasound machine. Each examination was performed by an obstetrics and gynecology specialist at Yasmin Clinic to count the number of follicles with diameter of 2 - 5 mm, with minimum 10 follicles as a sign of good ovarian reserve. The examinations were done in blinded fashion (without looking at medical records).

After all examinations had been done, IVF program was commenced, beginning with ovarian stimulation by recombinant FSH injection. Ovarian response was evaluated by counting the number of antral follicles with transvaginal USG done using ALOKA SSD 3500 ultrasound machine by an obstetrics and gynecology specialist at Yasmin Clinic during ovarian stimulation with recombinant FSH; serum E_2 levels were also measured. Ovarian response is defined as good if the transvaginal USG examination showed minimum three antral follicles with a diameter of $\geq$ 18 mm; and serum E_2 > 600 pq/ml. When good ovarian response had been achieved, hCG was administered. After 36 hours, ovum pick up was done. The final step was embryo transfer.

Two weeks after embryo transfer, 10 cc of venous blood was drawn for each patient at Yasmin Clinic and Makmal, Dr. Cipto Mangunkusumo Hospital. The first 5 cc was used to measure quantitave ß hCG level and ensure pregnancy, while the remaining 5 cc was used to measure AMH level post-ovarian stimulation. AMH levels measured were then compared to the levels pre-ovarian stimulation. Datas collected were documented on papers and analyzed with SPSS 12. After editing and coding, study data was burned on a magnetic disc for electronic data recording. Then, data was processed and prepared in distribution table to analyze mean, median, and distribution, in accordance with study aim.

RESULTS

Between January and July 2010, there were 56 new couples participating in IVF program at Yasmin Clinic, Department of Obstetrics and Gynecology, Dr. Cipto Mangunkusumo Hospital. Of the 56 patiens, 20 subjects were eligible for study.

Table 1. Distribution of study subjects by characteristics.

Characteristics	Subjects (n = 20)
Type of infertility (%)	
Primary	16 (80)
Secondary	4 (20)
Problem (%)	
Endometriosis	3 (15)
DOR	4 (20)
PCOS	3 (15)
Persistent Follicle	2 (10)
Bilateral Occlusion	2 (10)
Sperm abnormalities	6 (35)
Age (years)	35,3 ± 4,0
Duration of infertility (years)	9,1 ± 5,7
BMI (kg/m^2)	21,9 ± 3,4
FSH (mIU/ml)	7,5 ± 4,0
E_2 (pg/ml)	63,2 ± 61,3
FAB (follicle)	10,9 ± 8,9
Total FSH (IU)	2747,5 ± 1076,3
AMH pre- (ng/dl)	4,0 ± 4,1

Abbreviations: DOR = Diminished Ovarium Reserve; PCOs = Polycystic Ovary Syndrome.

To assess the effect of ovarian stimulation on AMH levels, a study was done to compare AMH levels prior to ovarian stimulation (AMH_1) and 3 weeks after ovarian stimulation on IVF (AMH_2). Data collected was tested with Shapiro-Wilk normality test, it was found that data distribution was not normal. Wilcoxon test was used to compare the median AMH_1 and AMH_2 levels. From the analysis, it was found that decrease in AMH level was not significant ($p = 0.295$) with following values; AMH_1; median 4.0 ng/ml, range 2.2 - 5.9 and AMH_2; median 3.2 ng/ml, range 1.6 - 4.8.

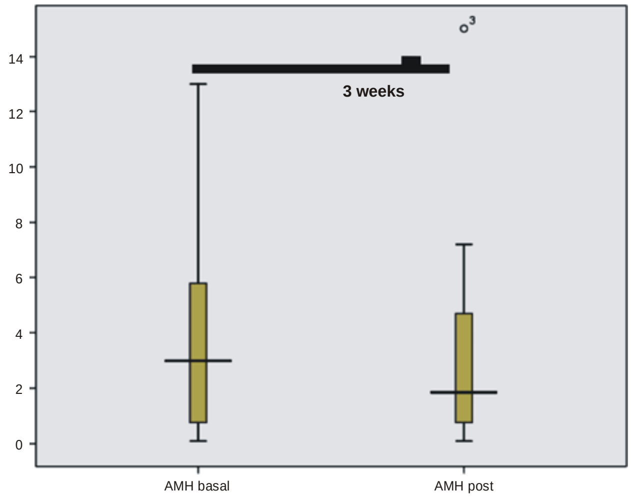

Figure 1. Comparison of AMH levels pre- (AMH_1) and 3 weeks post-stimulation (AMH_2). The line on the bars shows median, and (°) shows statistically insignificant decrease in ovarian reserve ($p > 0.05$).

DISCUSSION

According to Lee et al., 2010, the decrease happens because; first - FSH depress AMH expression, second - increase in estradiol during controlled ovarian stimulation will inhibit AMH secretion, and third - the reduction in the number of small antral follicle (there is a strong notion that AMH levels correlate with the number of small antral follicle).[10,11] AMH levels will rise again to levels before ovarian stimulation, probably due to decrease in exogenous FSH levels and subsequent loss of inhibition on AMH secretion. It can be said that this decrease is only temporary.

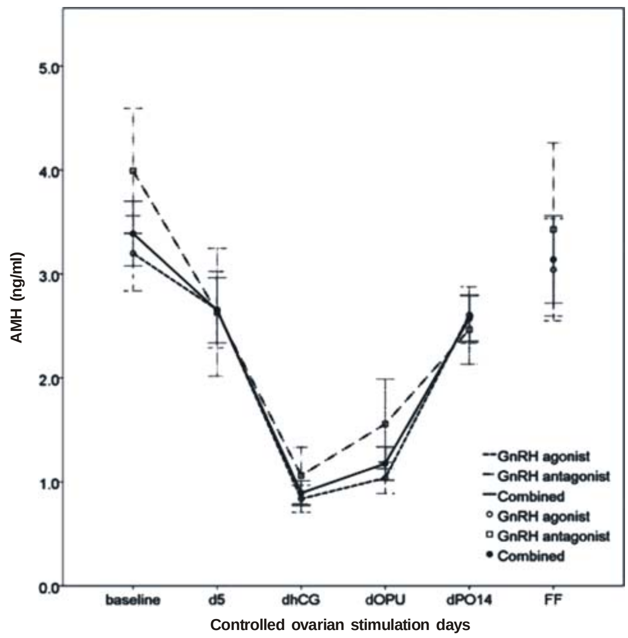

Figure 2. Fluctuation in AMH levels during controlled ovarian stimulation in IVF. Changes were significant ($p < 0.001$).[10]

Changes in AMH level could be observed, there was a significant decrease in AMH level on the day of hCG administration after ovarian stimulation with recombinant FSH, but this will eventually rise to base level after 2 weeks post embryo transfer or 1 month after ovarian stimulation. This AMH level dynamics described in the study by Lee et al, 2010, is consistent with the finding from this study which shows that AMH levels does not decrease significantly, this is because the AMH levels evaluated were AMH levels at base (pre-stimulation) and at 2 weeks after embryo transfer.[11]

Of the 20 subjects recruited, AMH decrease was found in 11 patients (55%), no decrease was found in the remaining 9 patients (45%). Variables from these two groups were compared in Table 2.

Table 2. Variables and its correlation with AMH level changes.

Variable	Decrease (n = 11)		No decrease (n = 9)		p
	Mean	SD	Mean	SD	
Age	33.3	3.2	37.8	3.5	0.007
Duration of infertility*	7.0	4.1	11.7	6.6	0.080
BMI	22.6	3.7	21.1	3.0	0.342
FSH*	6.0	2.3	9.1	4.9	0.156
E2*	61.7	67.9	165.1	56.6	0.965
FAB*	11,3	7,2	5,2	4,8	0,851
Total FSH*	2809.1	953.3	2672.2	1266.3	0.710
AMH pre*	6.5	4.1	1.0	1.1	0.000

**Mann Whitney formula*

The mean age of all patients was 35.3 years, the mean age of patients with AMH level decrease was 33.3 years with mean AMH_1 level of 6.5, and the mean age of patients with no decrease in AMH level was 37.8 years with mean AMH_1 level of 1.0. There were significant differences in both mean age and AMH_1 level of the two groups ($p = 0.007$ and $p = 0.000$). This leads to the notion that age and AMH levels pre-stimulation influence AMH levels post-ovarian stimulation.

In the group with younger age (< 36 years), there was a marked decrease of AMH levels post-ovarian stimulation. This may be owing to the higher AMH levels pre-stimulation. More decrease in AMH levels due to FSH-related inhibition was seen. Whereas, in the older aged group (> 36 years), AMH levels pre-stimulation were already low, so that the FSH-related inhibition effect was relatively less prominent.

Studies by de Vat and colleagues, Van Rooj and colleagues, and Wiweko and colleagues reported that age does have a strong correlation with AMH level.[10-12] The older a women is, the more AMH level will decrease, and this decrease happens before other parameters of ovarian reserve (FSH, E_2, Inhibin B and number of antral follicles) change.

CONCLUSION

AMH levels decrease following ovarian stimulation with recombinant FSH in IVF is not statistically significant and the process of ovarian stimulation in IVF has no effect on AMH levels as the decrease in AMH levels was found to be insignificant, however it is still unclear whether this stimulation does not affect ovarian reserve.

RECOMMENDATIONS

A follow up study - in a larger scale with serial AMH level monitoring done at more appropriate times during ovarian stimulation in IVF and post stimulation, is needed in order to evaluate ovarian reserve more accurately.

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