
SERUM ANTI- MULLERIAN HORMONE IS A BETTER PREDICTOR OF OVARIAN RESERVE AT THE EARLY FOLLICULAR PHASE.

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ABSTRACT

Objective : The study aim was to compare the relationship between serum anti-Müllerian hormone (AMH) levels and other markers of ovarian reserve and to determine its predictive value.

Design: prospective randomized trial .

Setting : Alexandria University and IVF-ICSI Madina private center .

Patients : 60 infertile women under going IVF-ICSI programmes.

Main Outcome Measure (s): Comparison of early follicular (day-3) serum AMH levels among women with less than five retrieved oocytes and five or more oocytes. Antral follicle count, mature oocyte count, age, basal follicle-stimulating hormone (FSH), inhibin B, estradiol (E₂), serum E₂ levels at day of hCG , and pregnancy success were also compared .

Result (s): The mean serum AMH levels of patients with more than five retrieved oocytes were significantly higher (0.67 ± 0.41 vs. 0.15 ± 0.11 pg/ml). Mature oocyte counts, antral follicle counts, and maximum E₂ levels were found to be statistically significantly different in the two groups despite the homogeneity of ages and levels of basal FSH and E₂.

Conclusion(s): This suggests that AMH may reflect ovarian follicular status better than the usual hormone markers.

Key words: anti- müllerian hormone (AMH) , ICSI.

INTRODUCTION

Anti-Müllerian hormone (AMH) is a glycoprotein hormone that belongs to the transforming growth factor superfamily⁽¹⁾ and is chiefly expressed in the fetal testis to drive differentiation of the mammalian reproductive tract⁽²⁾. In women, granulosa cell production of AMH⁽³⁾ is barely detectable at birth⁽⁴⁾ and reaches the highest values after puberty⁽⁵⁾. During adulthood, although AMH continues to be expressed at basal and similar levels by both the Sertoli and granulosa cells⁽⁵⁾, its biological role is poorly understood.

Basic research data obtained from the adult ovary indicate that AMH is likely to be involved in the

regulation of follicular steroidogenesis. Experiments conducted in animals suggest that AMH reduces aromatase activity and the number of LH receptors in FSH-stimulated granulosa cells⁽⁶⁾, and also influences testosterone production by theca cells⁽⁷⁾. In addition, growing evidence indicates that AMH is mainly expressed in pre-antral and early antral follicles⁽⁸⁾. It has either direct or indirect roles in various phases of folliculogenesis, from the primordial⁽⁹⁾ to FSH-sensitive^(10,11) follicular stages, probably via specific AMH type II receptors that are expressed in granulosa and theca cells. This suggests that, in contrast to other hormonal markers of ovarian function, AMH secretion might concurrently reflect the activity of pre-antral and early antral follicles, which makes it a promising parameter in the

evaluation of ovarian follicular reserve.

Recent clinical evidence is in agreement with this hypothesis. One group ⁽¹²⁾ demonstrated that serum AMH levels on cycle day 3 decrease progressively along with age and become undetectable after menopause. This suggests that peripheral AMH levels are a valuable parameter to monitor the relative follicular exhaustion due to ovarian ageing. Consistently, others ⁽¹³⁾ showed that day 3 AMH levels are positively related with the number of oocytes retrieved after controlled ovarian hyperstimulation (COH). Taken together, these results indicate that circulating AMH levels reflect the number of selectable follicles during the early follicular phase. Indeed, the early antral follicle count has been shown to reliably predict the fertility potential of women ⁽¹⁴⁾ and their responsiveness to COS ^(15,16).

However, the question of whether serum AMH measurements early in the follicular phase (on cycle day 3) reflect ovarian follicular status better than the usual hormonal parameters remains unanswered. Hence, the present investigation was planned to demonstrate the value of AMH as an indicator of ovarian reserve in comparison with other parameters of ovarian reserve (inhibin B, estradiol (E₂), FSH and LH early in the follicular phase or on cycle day 3).

MATERIALS & METHODS

Subjects

The study started in March 2007 by a total of 60 infertile women (age range 25-40 years). In order to be included, the women had to meet the following criteria: (i) regular, ovulatory menstrual cycles every 25-35 days; (ii) both ovaries present; (iii) no current or past diseases affecting ovaries or gonadotrophin or sex steroid secretion, clearance or excretion; (iv) a body mass index (BMI) of 18-27 kg/m²; (v) no current hormone therapy; and (vi) adequate visualization of ovaries at transvaginal ultrasound

scanning. All patients were undergoing routine explorations before IVF - ICSI - embryo transfer. Informed consent was obtained from all women.

Study protocol :

On day 3 of the menstrual cycle, each woman underwent blood sampling by venipuncture for measurement of serum levels of AMH, inhibin B, E₂, FSH and LH at approximately 09:00 h. Later in the morning, ovarian ultrasound scanning was performed using a 4.5-7.2 MHz multi-frequency transvaginal probe (new sonic, USA) by one operator (R.F.), who was blinded to the results of the hormone assays.

Long-protocol GnRH analogue was used for menstrual suppression. On cycle day 21, 0.5 mg/day of subcutaneous Buserlin acetate, was begun; E₂ levels less than 50 pg/mL on cycle day 2 and endometrial thickness less than 4 mm indicated down-regulation. Treatment with recombinant FSH, 150-300 IU/day, was started. Patients with serum E₂ levels less than 100 pg/mL and follicle count less than 3 on cycle day 5 were submitted to step-up protocol. Step-up or step-down protocols were decided according to subsequent ultrasound controls. In cases of less than two growing follicles or a serum E₂ level of less than 300 pg/mL on cycle day 14, treatment cycle was cancelled.

After more than three follicles larger than 18 mm were observed, 10,000 IU of hCG was administered intramuscularly, and 36 hours later follicles were aspirated under general anesthesia. Mature oocytes were retrieved from follicular fluid and placed in culture medium (G-1 TM version 3; Vitrolife, Goteborg, Sweden) prepared for ICSI and blastocyst development media (G-2 TM version 3; Vitrolife).

The study group was divided into two subgroups according to the number of oocytes retrieved. Patients with an oocyte count of five or more were considered good responders, and patients with less than five as poor responders. The 30 patients being treated for male factor infertility received ICSI; and IVF - ICSI

was provided for the 23 patients with other infertility causes. In ten patients, cycles were cancelled for economic reasons. The transfer of the embryos was performed 48 to 72 hours after the procedure. Before transfer, the embryos were evaluated microscopically to ensure six to eight cells in each embryo of grades 1 to 2. A maximum of three embryos was transferred. On the 12th to 14th days of the transfer, a serum β -hCG test was performed to confirm pregnancy.

Statistical analysis

Statistical analysis were performed using the χ^2 , or t-test as appropriate. All P-value quoted with values < 0.05 indicate statistical significance. Analysis were performed using the SPSS statistical package (SPSS, Inc, Chicago)

Hormone measurements

Serum was separated from all blood samples and frozen in aliquots at -20°C until used for subsequent centralized analysis. Serum AMH levels were determined using an ultrasensitive enzyme-linked immunosorbent assay (ELISA) (Beckman-Coulter, Villepinte, France) as described previously (17). For AMH, functional sensitivity was 0.24 pg/ml. Serum inhibin B levels were determined using a double antibody ELISA (Serotec, Varilhes, France) as described previously(18). For inhibin B, functional sensitivity was 15 pg/ml. Serum levels of E_2 , FSH and LH were determined using an automated multi-analysis system with chemiluminescence detection (ACS-180; Bayer Diagnostics, Puteaux, France). For E_2 , functional sensitivity was 15 pg/ml.. For FSH and LH, functional sensitivity was 0.1 mIU/ml..

RESULTS

The study included 60 patients in the IVF-ICSI program. Ten patients dropped out of the study for economic reasons. Six patients' AMH levels could not be determined for technical reasons. Of the remaining 44 couples, the causes of infertility were male factor (25 couples), tubal factor (seven couples),

and idiopathic (12 couples). The ages of the female patients were between 24 and 39 years. The mean infertility period was 9 ± 3.9 years, and the mean body mass index of female patients was in the normal range (see Table). Ovarian reserve was assessed by basal hormone levels on cycle day 3.

Treatment was started with a mean dose of 244 ± 59 IU/day of recombinant FSH (150-300 IU); a total dose of 2410 ± 978 IU/day (1050-5400 IU) was reached. A mean period of 9 ± 2 days (5-14) was spent on the treatment. The results of the study were evaluated according to the number of growing follicles, oocytes, and mature oocytes retrieved (see Table I). Fifteen couples ended in pregnancy. Two of them continued as twin pregnancies, and eight as singletons. Three pregnancies were aborted, and two were biochemical pregnancies.

The study group was divided into two subgroups according to the number of oocytes retrieved. Patients with an oocyte count of five or more were considered good responders and less than five as poor responders. All parameters were compared for these two groups. Characteristics such as age, infertility period, and body mass index were similar between good and poor responders.

Good responders had a mean beginning dose of 249 ± 58 IU/day, and a total dose of 2363 ± 969 IU was reached in 8 ± 2 days. Poor responders had a mean starting dose of 231 ± 62 IU/day, and a total dose of 2550 ± 1041 IU was reached in a mean period of 9 ± 1 days. These parameters were statistically alike. Parameters such as basal serum FSH, inhibin B and E_2 levels were also not statistically different. Meanwhile, the difference between serum AMH levels, maximum E_2 levels, antral follicle count, hCG day follicle counts, and mature oocyte counts were statistically significant (see Table I).

The main parameter of the study, AMH, was found to be considerably higher in good responders; mean level was 0.67 ± 0.41 pg/mL. The AMH level was 0.15 ± 0.11 pg/mL in poor responders ($P < .001$).

Table I:

Parameters	Poor responders n=11	Good responders n=33	P value
Age	32.63 ± 3.58	30.75 ± 4.10	NS
Infertility period	12.0 ± 4.17	8.0 ± 3.37	NS
Body mass index	24.68 ± 5.14	24.69 ± 2.94	NS
Day-3FSH (mIU/ml)	7.35 ± 3.49	7.53 ± 2.23	NS
Day-3 E2 (pg/ml)	36.40 ± 22.86	33.16 ± 9.83	NS
Day-3LH (mIU/ml)	8.44 ± 11.17	5.40 ± 2.58	NS
Day-3 AMH (pg/ml)	0.15 ± 0.11	0.67 ± 0.41	P < 0.001
Day 3 Inhibin B (pg/ml)	16 ± 182	18 ± 174	NS
Antral follicle count	6.90 ± 3.41	10.63 ± 3.89	P < 0.001
hCG day E2 (pg/ml)	1252.54 ± 605.29	2584.81 ± 927.02	P < 0.001
Follicle count	6.09 ± 2.42	11.66 ± 3.01	P < 0.001
Retrieved oocyte count	3.54 ± 1.21	10.69 ± 3.47	P < 0.001
Mature oocyte count	2.6 ± 1.2	9.21 ± 3.68	P < 0.001

DISCUSSION

The present investigation was designed to evaluate the direct relationship between peripheral AMH levels, measured by an ultrasensitive ELISA technique, and the ovarian follicular status on cycle day 3.

Our data demonstrate an association between early follicular phase serum AMH and number of retrieved oocytes despite clinically similar day-3 serum FSH and E₂ levels in patients of all age groups. Baseline FSH, LH, inhibin B and E₂ levels are good predictors of ovarian reserve^(19, 20). As noted by other studies, Jurema et al.⁽²¹⁾ found that levels of baseline FSH and E₂ but not LH were statistically significantly lower in cycles resulting in a normal ovarian response as well as cycles resulting in clinical pregnancy. It was also stated that a stricter FSH value (8 IU/mL) would have been required for the group of patients with poor prognosis^(19,20,21). In our study, good and poor responder groups had similar mean baseline FSH levels (lower than 8 IU/mL), which gave us an opportunity to underline

the importance of AMH and the relation between AMH and the number of retrieved oocytes.

Bancsi et al.⁽²²⁾ found that antral follicle count is the best single indicator for ovarian response. The combination of antral follicle count with basal FSH and inhibin B levels improved prediction. Van Rooij et al.⁽²³⁾ combined antral follicle count with AMH and inhibin B to provide for better prediction. Fanchin et al.⁽²⁴⁾ found that AMH levels correlated more with the number of early antral follicles than did inhibin B, E₂, FSH, or LH on cycle day 3.

Expression of AMH begins in the third trimester of gestation, long before it can be detected in serum. As a result, it is thought to play an important role in early follicular development. Once menstrual cycles begin, serum AMH levels fluctuate slightly from baseline due to the stimulation of a small cohort of follicles and loss of AMH production from corpus luteum^(25,26). Our results demonstrated an association among AMH and antral follicles and retrieved oocyte count; so serum AMH levels may reflect the size of antral follicle pool. It may thus provide a marker associated with the anticipated

number of oocytes to be retrieved after controlled ovarian stimulation. Early follicular phase AMH levels result from the pool of follicles that began producing it in utero, and this production is independent of the gonadotropin-dependent indicators of ovarian reserve; this makes AMH unique in providing a perspective not available by using current serum markers and ultrasound⁽¹³⁾.

Thus, basal antral follicle count and basal AMH levels are good tools for use in counseling patients^(27-13,22,24). However, accurate antral follicle counts depend on the clinician's experience and the technical properties of the ultrasound used. By contrast, AMH levels are obtained by objective measurements performed in laboratory medium and thus are free of interobserver variability and personal comments.

CONCLUSIONS

Our data indicate that early follicular AMH levels can offer a contribution to the serum markers currently being used for evaluating ovarian reserve, but AMH levels will not reveal the possibility of pregnancy.

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