Original Article

Evaluation of Ovarian Reserve by Measurement of the Serum Levels of Anti-Mullerian Hormone and Follicle-Stimulating Hormone in Intracytoplasmic Sperm Injection Cycles

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Abstract

Background: It is important to evaluate ovarian reserves prior to intracytoplasmic sperm injection (ICSI) treatment. The aim of this study is to determine the accuracy of anti-mullerian hormone (AMH) as a marker for ovarian reserve and to compare it with day-3 serum follicle-stimulating hormone (FSH) levels.

Materials and Methods: In this analytic, corss-sectional study, sequential sampling was done on 70 infertile women who underwent ICSI treatment at Imam Khomeini Hospital, Ahvaz, Iran.
Initially, 5cc of venous blood was drawn from each patient to measure serum AMH and FSH levels on the day-3 cycle.
Patients were divided into two subgroups according to the numbers of oocytes retrieved. Patients were classified as good responders if there were four or more oocytes retrieved, whereas patients with less than four oocytes were poor responders.

Results: The basal AMH level correlated with the number of oocytes retrieved (linear Pearson correlation coefficient=0.599), however the basal FSH level had a weakly reverse correlation (correlation coefficient = -0.11).
AMH levels had a sensitivity of 85% and specificity of 61.5%, with a cutoff value equal to 2.3 ng/ml which was higher than FSH.

Conclusion: AMH serum levels are good predictors of ovarian reserve in comparison with FSH.

Keywords: AMH, FSH, ICSI

Introduction

Determination of ovarian reserve is important in *in vitro* fertilization (IVF) treatment. Identification of both low and good responders prior to treatment may decrease the cycle cancellation rate and side effects such as ovarian hyper-stimulation syndrome.
Determination of the probability of pregnancy is important in the cycles which allows physicians to evaluate and counsel patients prior to IVF treatment and if necessary, allows the patients to consider other treatments such as gamete donation or adoption (1).
The other markers for ovarian reserve include age, basal follicle-stimulating hormone (FSH), estradiol and serum inhibine B levels, antral follicle counts, ovarian volume and vascular resistance (2). However, basal FSH levels are not useful predictors of IVF outcome which is probably due to inter-cycle variability. In several studies, serum inhibine B has shown no or limited clinical value (1).
Antral follicle count is dependent on clinician's experience.
Provocative and dynamic tests such as the gonadotropin releasing hormone agonist (GnRH agonist) and clomiphene citrate tests have been introduced recently, but they needs to interventions (2).
A new suggestive marker is the dimeric glycoprotein anti-mullerian hormone (AMH), also termed mullerian-inhibiting substance which acts on tissue growth and differentiation.
AMH is secreted from pre-antral and early antral follicles, and regulates ovarian activity and follicular steroidogenesis. AMH plays a major role in the regulation of the intrafollicular androgen to estrogen ratio (2).
AMH is not measurable until puberty in females, after which it decreases progressively until it becomes undetectable around the time of menopause (2, 3).
At present, AMH levels are not considered part of a routine infertility work up. Although they have been associated with greater numbers of retrieved oocytes in IVF cycles and with improved embryo morphology, but

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it has not yet been reported (4).
The aim of this study is to investigate the correlation of serum AMH and FSH levels in women undergoing ICSI treatment and their outcomes.

Materials and Methods

This was an analytic cross-sectional study of 70 women who underwent intracytoplasmic sperm injection (ICSI) at Jundishapur University of Medical Science, Ahwaz, Iran from January 2008 until January 2009.
Patients' medical histories were recorded. All patients underwent general physical and gynecologic examinations, in addition to pap smears, count blood cell (CBC), blood group and Rh type, serologic tests (HBsAg anti-HCV Ab, anti-HIVAb), hysterosalpingography, thyroid function test and serum prolactin. Additionally, patients' partners were requested to undergo spermogram, CBC and serologic tests.
This study was approved by the Ethical Academic Board of Jundishapor University.
Women were included in the study if they had a history of regular menstrual cycles (21-35 days), body mass index (BMI) less than 30 kg/m^2, normal uterine anatomy with the presence of any tubal disease except for distal occlusion and hydrosalpinx as seen by hysterosalpingography, and patients' partners were not azoospermic.
Patients who were smokers were excluded from the study. After obtaining patients' informed consents, 5 ml of blood was drawn on the third day of their cycles. The blood was centrifuged at 3500 cycles/minute for 10 minutes,and the sera was stored at -20°C. Subsequently, the levels of serum AMH were measured with an A16507 kit (Immunotech, Beckman Coulter Company) and FSH levels were determined by FSH ELISA (Mono bind), USA).
In all patients treated by the ICSI protocol, a low dose oral contraceptive pill commenced on days 3-5 of the previous cycle and a dose of 50 µg GnRh agonist (Buserlin, Suprefact, Aventis Pharma, Germany) was begun on days 19-21 of the previous cycle. On days 1-3 of the cycle, transvaginal ultrasonography was performed. If a follicular size of less than 15 mm and endometrial thickness less than 5mm were observed, the dose of GnRH agonist was decreased to half and human gonadothropin (HCG) at a dose of 225-300 IU/day (IM or SC) was initiated. When more than two follicles larger than 17-18 mm were observed, 10000 IU HCG was administered IM and 34-36 hours later, ovum pickup was performed by transvaginal ultrasonography. If the response of the ovaries was less than two mature follicles, the cycle was cancelled after counseling with the patient. The oocytes were incubated for approximately 4 hours after retrieval, then ICSI was done.
Embryo transfer was performed 72 hours after this procedure. Before transfer, the embryos were evaluated microscopically for determination of their grade.
The embryos were transferred 1.5-2 cm of the uterine fundus with a Labotec catheter. The maximum number of embryos transferred was three. On the 14th day after transfer, a serum β-hCG test was performed to confirm pregnancy (5-7).
The study group was divided into two subgroups, according to the number of retrieved oocytes (as a gold standard for success rate). Patients with four or more oocytes were considered good responders and patients with less than four as poor responders (8). Statistical analysis was performed with SPSS 15.
Specificity and sensitivity were calculated for both the AMH and FSH levels. Pearson coefficient correlation was analyzed with the student's t-test. $P<0.05$ was considered statistically significant.

Results

This study included 70 patients who underwent ICSI treatment. Three patients dropped out of the study due to economic reasons.
Descriptive data of the patients and the serum levels of AMH, FSH, the number of retrieved oocytes, the number of embryos and dose of HMG are shown in table 1.

Table 1: Demographic data

	Min	Max	Mean
Age of women (years)	21	42	30.76
Age of men (years)	23	54	35.7
AMH (ng/ml)	0.01	8.5	4.30
FSH (ng/ml)	0.5	19	8.34
Retrieved oocytes(n)	0	30	10.24
Embryos(n)	0	11	3.42
HMG (n) 75 IU/vial	14	69	33.12

Tables 2 and 3 show the correlation between the numbers of oocytes retrieved with the two tests. It was determined that basal AMH levels statistically correlated with the number of retrieved oocytes as well as the number of embryos, but there was a reverse correlation with the dose of HMG.

Table 2: Linear Pearson coefficient correlations Between AMH & (oocyte, embryo and HMG)

	Correlation coefficient	P-value
AMH and oocytes	0.599	* 0.001
AMH and embryos	0.258	* 0.035
AMH and HMG	-0.376	* 0.002

Statistically significant

Table 3: Linear Pearson coefficient correlation Between FSH & (oocyte, embryo and HMG)

	Correlation coefficient	P-value
FSH and oocytes	-0.119	0.336
FSH and embryos	-0.150	0.225
FSH and HMG	0.274	*0.025

Statistically significant

The linear Pearson correlation coefficient between AMH and the oocytes retrieved was 0.59 (p =0.001). There was a positive weak correlation between the basal FSH level and the dose of HMG and a weak reverse correlation with the number of oocytes retrieved and the number of embryos.
The serum level of AMH had a sensitivity of 85.19% and specificity of 61.54% with a cutoff point of 2.3 ng/ml. Serum FSH levels had a sensitivity of 74.04% and a specificity of 38.46% with a cutoff point of 2.3 ng/ml.

Discussion

In our study we found that the measurement of serum AMH levels may be useful for the accurate assessment of ovarian reserves, although it was not completely accurate in predicting the success or failure of ART cycles. AMH correlated better with ovarian reserves than basal FSH levels, which is in contrast to some previous studies that reported a reverse correlation between serum AMH and FSH levels (2, 4, 9-11). The serum level of AMH had a sensitivity of 85.19% and specificity of 61.54% at a cut off point that was equal to 2.3 ng/ml, which was higher than the FSH level. Therefore our result is relatively compatible with previous studies (10, 12-19).
Riggs et al. have reported that AMH correlates with the number of retrieved oocytes in comparison with age, FSH, LH, inhibine B and estradiol (10).
In the study that was performed by Elder - Geva et al. the only predictor for pregnancy was follicular or luteal phase AMH (1).
The previous studies note AMH to be relatively stable throughout the menstrual cycle and unaffected by exogenous or endogenous hormonal perturbations, although further studies are critical to confirm these observations (20, 21).
The studies initially suggest that AMH has a limited inter-cycle variability, however FSH has both inter- and intra-cycle variability and occurs late in the reproductive aging process, which limits their accuracy for the prediction of ovarian responsiveness to fertility therapy (22-24).
Visser et al. have reported that serum levels of AMH correlate strongly with the number of antral follicles, which suggested that AMH levels reflect the size of the primordial follicle pool (25).
Fanchin et al. reported that AMH may reflect ovarian follicular status better than the usual hormone markers (3).
Additionally, Silberstein has reported that the serum level of mullerian inhibiting substance could predict not only ovarian reserve, but also embryo morphology (9).

Conclusion

We suggest another study be undertaken that utilizes a larger number of patients than previous studies in order to document AMH levels as a useful marker in an infertility workup, particularly in poor responders.

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There in no conflict of interest in this article.

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