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The association between anti-Müllerian hormone and IVF-ICSI outcome in poor responder patients performing long protocol

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Summary

Purpose of investigation: Ovarian reserve reflects the capacity of the ovaries for a successful pregnancy. Anti-Müllerian hormone (AMH) could be a useful marker to predict ovarian reserve and to adjust controlled ovarian stimulation. The aim of this study was to assess the relationship between AMH and intracytoplasmic sperm injection-in vitro fertilization (IVF-ICSI) outcome in poor responder women. *Materials and Methods:* This study was conducted prospectively for a period of 12 months. Inclusion criteria were FSH value > 15 iu/l or antral follicle number < 4, on the 2nd day of cycle. All patients underwent GnRH agonist stimulation with long protocol. Serum AMH levels were measured in the treatment cycle just before the stimulation. After the treatment, patients who were pregnant formed the study group and patients who were not pregnant formed the control group. Serum AMH level was the main outcome measure. *Results:* The study and control group consisted of 34 and 70 patients, respectively. No significant difference was found in duration of infertility, antral follicular count, basal E_2 and FSH levels. The mean serum AMH level was significantly higher in study group (p = 0.005). The retrieved oocyte number, metaphase 2 oocyte number, and fertilization rate were also significantly higher in the study group. *Discussion:* Evaluation of serum AMH seems to be a useful marker to predict IVF-ICSI outcome in poor responder patients.

Key words: IVF-ICSI; Anti-Müllerian hormone; Poor responder; Ovarian reserve.

Introduction

Intracytoplasmic sperm injection-in vitro fertilization (IVF-ICSI) is associated with yielded oocytes' number and quality. Poor responder patients are one of the most important problems in IVF-ICSI management. Success of the treatment decreases in such patients [1]. Evaluation of ovarian reserve could help to predict the patients who will not become pregnant. Thus, the duration of treatment and the management of controlled ovarian hyperstimulation (COH) might be easily performed. Numerous tests and methods have been available to assess ovarian reserve. However, there is no ideal test demonstrating ovarian reserve and pregnancy estimation [2]. Among these tests only measurement of antral follicle count (AFC) and serum anti-Müllerian hormone (AMH) levels have found to be beneficial. Broer *et al.* reported that AFC and AMH were useful to predict poor response [3]. But both of them were not successful to estimate a pregnancy.

AMH is a dimeric glycoprotein produced by granulosa cells in pre-antral and antral follicles [4, 5]. Serum AMH levels remain unchanged in conditions in which endogenous gonadotropin levels are low such as with pregnancy, ovulation induction or treatment with oral contraceptive pills [6]. An ideal ovarian reserve test should rapidly assess the chance of pregnancy. Therefore, the cost of the treatment would decrease due to inclusion of convenient patients into assisted reproductive treatment (ART). Although AMH is the best marker demonstrating poor response, there is no cut-off value for the patients whether to start a treatment in relation with IVF or not [7]. Thus, this study was designed to detect the association between serum AMH levels and IVF-ICSI outcome and to determine a cut-off level for AMH to predict IVF-ICSI success.

Materials and Methods

This study was conducted prospectively for a period of 12 months. The study protocol of this clinical trial was reviewed and approved by the Ethical Committee of Bozok University Medical Faculty. Informed consent was taken from all participants. Inclusion criteria for poor responders were FSH value > 15 iu/l or antral follicle number < 4, on the 2nd day of cycle. Serum AMH levels were measured in the treatment cycle just before the stimulation. After the treatment, patients who were pregnant formed the study group and patients who were not pregnant formed the control group.

Controlled ovarian hyperstimulation (COH)

Pituitary was down-regulated with one mg/day leuprolide acetate. It was given for at least ten days beginning on the 21st day of the previous menstrual cycle. On the second day of menstruation, transvaginal ultrasonography (TVUSG) and serum E_2 levels were monitored to show the suppression. When the serum E_2 level

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Table 1. — *Characteristics of the patients.*

	Not pregnant (n=70)	Pregnant (n=34)	*p*	95% CI
Age (years)	32.12 ± 6.01	29.14 ± 3.96	0.01	-0,72 to 5,23
DI (years)	6.29 ± 0.63	7.20 ± 1.02	0.38	-0,42 to 1,27
Basal E_2 level (pg/ml)	63.04 ± 13.31	58.93 ± 11.27	0.95	9,25 to 21,66
Basal FSH level (iu/l)	19.31 ± 0.71	18.73 ± 0.62	0.44	-1,33 to 2,85
AFC	3.04 ± 0.46	2.76 ± 0.29	0.56	-0,35 to -0,77

Note: Values are expressed as mean ± SD.
CI: confidence interval; DI: duration of infertility;
FSH: follicular stimulating hormone; AFC: antral follicle count.

was below 50 pg/ml, COH was started with highly purified-urinary FSH on the 2nd day of the cycle. Then GnRH agonist dose was decreased to 0.5 mg/day. The beginning FSH dose was 300 iu and the dose was individually adjusted according to the previous treatment cycles, body mass index (BMI), and age. COH was monitored by using E_2 measurement and TVUSG. Human chorionic gonadotropin (hCG) (5,000 IU × 2) was administered when the dominant follicle reached 17 mm. Oocytes were retrieved by TVUSG-guided needle aspiration. IVF-ICSI was performed in all cases. Luteal phase support was given by vaginal progesterone (8% vaginal gel) beginning on the oocyte pick-up (OPU) day and lasted for 12 days (until the serum β-hCG measurement day). If pregnancy occurred, progesterone was given until the 12th gestational week. Clinical pregnancy was accepted as a pregnancy when the gestational sac or fetal heartbeat was confirmed by TVUSG.

Statistical analysis

Statistical analysis was performed using SPSS 17.00. The Chi-square test was used for categorical variables, independent sample t-test was used for continuous variables, and Mann Whitney U test was used to compare median values. A *p*-value < 0.05 was considered significant. The area under the receiver operating characteristic curve was used to determine discriminative power of serum AMH level in prediction of IVF/ICSI outcome.

Results

One hundred and four women were included the study. All patients were on their first cycle of IVF-ICSI treatment. The long protocol was well tolerated by the patients. No systemic adverse effects were observed and no severe ovarian hyperstimulation syndrome (OHSS) occurred. After the treatment 34 patients (32.6%) became pregnant and formed the study group. Control group consisted of seventy patients (67.4%), pregnancy could not be achieved.

In control group, the mean age was 32.12 ± 6.01 years and it was significantly higher than study group (*p* = 0.01). No significant difference was found in duration of infertility, antral follicular count, basal E_2, and FSH levels (Table 1). The mean serum AMH level was significantly higher in study group (*p* = 0.005). The retrieved oocyte number, metaphase 2 oocyte number and fertilization rate were also significantly higher in study group. Comparison of the groups in relation with IVF-ICSI outcome is shown in Table 2.

Table 2. — *Comparison of IVF-ICSI outcome according to the pregnancy status.*

	Control group (not pregnant, n=70)	Study group (pregnant, n=34)	*p*	95% CI
IN	1<1-3>	1<1-4>	0.402	
RON	4.71±3.55	6.67±2.85	0.006	-3,34 to -5,76
MON	3.34±2.46	5.26±2.53	0.001	-2,95 to -0,89
FR, (%)	(48.9)	(57.86)	0.001	
AMH	0.45±0.28	0.61±0.23	0.005	-0,264 to -0,047
E_2 on hCG day	1949.55± 2264.62	2264.21± 2680.64	0.53	-1312,70 to 683,40
P on hCG day	0.65±0.22	0.69±0.21	0.48	-0,123 to 0,059

Note: Values are expressed as mean ± SD, (percentages) and median <range>.
IN: intervention number; CI: confidence interval; RON: retrieved oocyte number; MON: metaphase 2 oocyte number; FR: fertilization rate; P: progesterone.

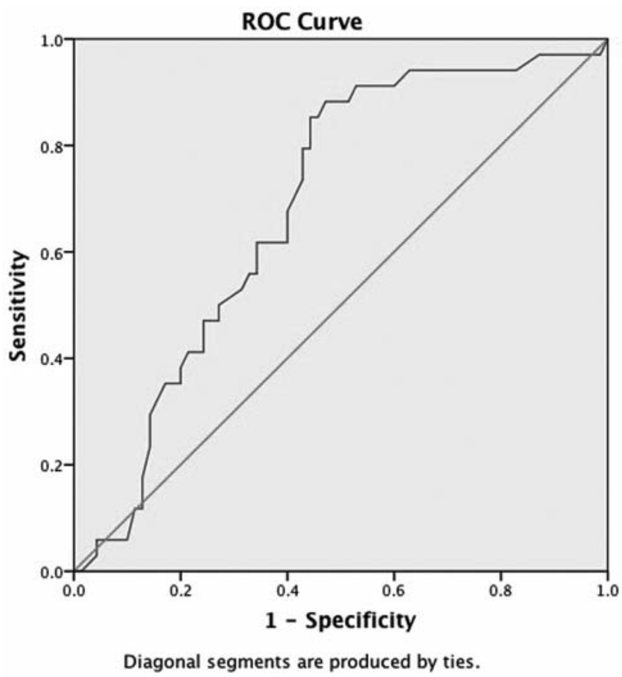

Figure 1. — The ROC analysis of AMH. Area under the curve for AMH: 0.686. Cut off value: 0.4 (sensitivity: 0.85, 1-specifity: 0.44.

Area under the curve for AMH: 0.686 (Figure 1). The cut-off value for AMH was 0.4 ng/ml (sensitivity 85%, specificity 44%). The cumulative ongoing pregnancy rate (OPR) was 24% (25/104) with this cut-off level. The cumulative ongoing pregnancy rate (OPR) was 24% (25/104) with this cut-off level.

Discussion

In this clinical study, the association between serum AMH levels and IVF-ICSI outcome was evaluated. Also, a cut-off level for AMH was attempted to predict the success of IVF-

ICSI treatment. To the best of the present authors' knowledge, there are few studies available demonstrating the relationship between serum AMH level and cumulative pregnancy outcome. This study has demonstrated that, serum AMH level measurement seems to be a useful marker to predict IVF-ICSI success in poor responder patients. The cut-off value for AMH was found to be 0.4 (sensitivity 85%, specificity 44%).

Poor ovarian reserve and advanced maternal age are the most important factors influencing the success of IVF-ICSI. Management of COH and administration of convenient protocol are critical. Therefore, prediction of a poor response status helps to individualize the treatment regime and to yield the best results [8]. Numerous static tests such as FSH, inhibin β, ovarian volume, AFC, or dynamic test as clomiphene citrate challenge test (CCCT), GnRH agonist stimulation test (GAST), and exogenous FSH ovarian reserve test (EFORT) have been utilized in the past [6, 7, 9]. However, the values of these tests are limited.

AMH has been introduced as a novel marker predicting ovarian reserve [9-11]. Hazout *et al.* reported that 1.1 ng/ml was a cut-off level for AMH. They founded that IVF-ICSI outcome was poor with an AMH level less than 1.1 ng/ml. Also, AMH measurement was found to be more prognostic than age, serum FSH, inhibin B or estradiol [9]. La Marca *et al.* demonstrated that AMH level remains unchanged throughout menstrual cycle. However, other steroids secreted from ovaries exhibit important variability [11]. Streuli *et al.* showed that AMH levels exhibit alterations during the cycle [12], but these fluctuations were found to be slight and clinically negligible. Therefore, serum AMH levels were accepted as stable during the cycle when they compared with other ovarian reserve markers.

Various cut-off levels were described for AMH. Buyuk *et al.* reported that IVF-ICSI outcome was better with random serum AMH levels ≥ 0.6 ng/ml [13]. Gleicher *et al.* identified an AMH cut-off of 1.06 ng/ml [14]. They affirmed that poor responder patients could have increased pregnancy rates with an AMH level > 1.06 ng/ml. Celik *et al.* detected that an AMH cut-off level ≥ 1 ng/ml had a sensitivity of 58.7% and specificity of 95.1% in poor responder women [15]. In the present study, the authors found an AMH cut-off level ≥ 0.4 ng/ml (sensitivity 85%, specificity 44%). The patients had basal FSH level > 15 iu/ ml and AFC < 4.

In conclusion, the present authors analyzed the association between serum AMH level and IVF-ICSI outcome. The pregnancy rates were significantly lower with AMH levels < 0.4 ng/ml. AMH was found to be a useful parameter to predict poor ovarian reserve. Therefore, measurement of serum AMH level will take its rightful place for predicting the success of IVF-ICSI in near future. However, large prospective randomized controlled studies are required to show the importance of AMH in predicting ovarian reserve and IVF-ICSI outcome.

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