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ASSISTED REPRODUCTION TECHNOLOGIES

Anti-Müllerian hormone levels as a predictor of the pregnancy rate in women of advanced reproductive age

Sezai Sahmay · Mahmut Oncul · Abdullah Tuten · Abdullah Tok · Abdullah Serdar Acıkgoz · Ismail Cepni

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Abstract
Purpose To investigate whether serum anti-müllerian hormone (AMH), follicle stimulating hormone (FSH), or antral follicle count (AFC) are predictive for clinical pregnancy in women who underwent IVF cycles at the age of 35 and older
Methods A total of 240 consecutive women who underwent IVF cycles at the age of 35 and older were enrolled in this crsoss- sectional study. Pregnant and nonpregnant women were compared.
Results The median AMH level of pregnant women was higher than non-pregnant women [3.20 (0.63–9.60) vs 1.15 (0.01–14.90) ng/ml, $p<0.001$]. On logistic regression analysis, AMH was an independent predictor of clinical pregnancy rate (CPR) (OR 1.353; 95 % CI 1.141–1.605; $P<0.001$). After controlling for the other independent variables (the number of retrieved oocytes, AFC and age), the significant association between AMH and clinical pregnancy rate remained strong (OR 1.677; 95 % CI 1.216–2.311; $p=0.002$) on multivariate logistic regression analysis.
Conclusions AMH is an effective measure of quantitative ovarian reserve and it can predict ovarian response to controlled stimulation for advanced age women. The CPR tends to increase as AMH increases.

Keywords Anti-Müllerian hormone · Advanced reproductive age · In Vitro Fertilization

Capsule The AMH levels are more useful than FSH and AFC for the prediction of clinical pregnancy rate in women who underwent IVF cycles at the age of 35 and older.

S. Sahmay · M. Oncul · A. Tuten · A. S. Acıkgoz · I. Cepni
Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey

A. Tok
Department of Obstetrics and Gynecology, Mersin Public Hospital, Mersin, Turkey

M. Oncul (✉)
Department of Obstetrics and Gynecology,
Cerrahpasa Medical Faculty, Istanbul University,
Kizilelma Caddesi, No: 35/1, Fatih, 34300 Istanbul, Turkey
e-mail: mahmutoncul@gmail.com

Introduction

It is well known that with increasing chronological age, female fecundity decreases. Due to the general tendency to postpone childbearing, an increasing proportion of couples will depend on assisted reproductive technology (ART) to achieve a pregnancy. Etiology of infertility, age, duration of infertility, the quality and quantity of oocytes are all basic determinants for the success in ART [1].

Numerous tests such as follicle stimulating hormone (FSH) [2], estradiol [3], inhibin B [4], and antral follicle counts (AFC) [5], have been suggested to improve prediction of oocyte yield and pregnancy outcome following in ART.

Biochemical markers have limited use since they have a low predictive value, show cycle-dependent variations, may be subject to disparities between laboratory assays, and lack clear cut-off values. And, antral follicle count (AFC) has been shown to be affected by interobserver variation [6–9].

A relatively new marker, anti-müllerian hormone (AMH) was evaluated by several study groups as a marker of ovarian response [10, 11]. AMH, a member of the transforming growth factor-beta super-family, is mainly secreted by the granulosa cells of ovarian early developing follicles. AMH levels are shown to be age dependent [12] and AMH is becoming widely accepted as a reliable clinical marker of ovarian reserve, since the relationship between the number of primordial follicles and their rate of activation, which is reflected in the number of growing follicles is documented [13].

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Although some studies have shown that AMH could be a predictor of ovarian reserve and the success rates of IVF [14, 15], others could not find to predict power of pregnancy outcomes [16, 17]. In our previous two studies on AMH and pregnancy prediction, we were unable to show AMH as being a good pregnancy prediction marker in both normoresponder and polycystic ovary syndrome (PCOS) groups [18, 19].

The aim of this study was to investigate whether serum AMH, FSH, or AFC are predictive for clinical pregnancy in women who underwent IVF cycles at the age of 35 and older.

Materials and methods

Between March 2010 and August 2013, a total of 240 consecutive women who underwent first IVF cycles at the age of 35 and older were enrolled in this retrospective cross- sectional study. All of the patients were admitted to the Istanbul University, Cerrahpasa School of Medicine, Department of Obstetrics and Gynecology, IVF Center for the IVF treatment.

The initial inclusion criteria were: (1) >35 years of age, (2) both ovaries present on transvaginal ultrasound scan, (3) no previous history of ovarian surgery. The exclusion criteria for this study was: current or past diseases such as hepatic, renal, adrenal or thyroid disorders, hyperprolactinemia and any condition affecting ovaries or gonadotropin or sex steroid secretion, clearance, or excretion.

This study was approved by the Istanbul University Cerrahpasa medical faculty′s ethical committee. Blood samples were collected during the early follicular phase of menses in all women. AMH, FSH, luteinizing hormone (LH), E2, PRL, Inhibin-B and TSH were measured in all women. All blood samples for measurements of AMH levels were collected in a lithium heparin tube and were stored at−80 ° Celcius AMH concentrations were measured with an enzymatically amplified two-sided immunoassay [AMH Gen II ELISA, Beckman Coulter Inc Brea, CA]. The theoretical sensitivity of the method is 0.006 ng/ml, the intra-assay coefficient of variation for high values is 3.3 %, and the interassay coefficient of variation for high values is 6.7 %.

Controlled ovarian stimulation was performed with a gonadotropin-releasing hormone (GnRH) antagonist protocol, a long GnRH agonist protocol and a short GnRH agonist protocol. In our clinic, agonist and antagonist protocols were chosen according to the clinician′s choice and the patient′s preference.

Patients with 0–3oocytes retrieved were evaluated as poor responders.

A transvaginal ultrasound scan was arranged on days 7 and 9 of ovarian stimulation and every 1 or 2 days thereafter, as required. The dose of the gonadotropin was changed according to the follicular growth. When more than 2 follicles were seen that were >17 mm, hCG (Pregnyl®, 10,000 IU, Schering Plough, Istanbul or Ovitrelle® 250 mcg, Serono, Swiss) was injected to induce final oocyte maturation, and 36 h later, ovum pick-up (OPU) was performed. The embryos were transferred after 3 or 5 days if fertilization had occurred. The luteal phase was supported with progesterone 90 mg administered by the vaginal route once or twice a day (Progynex® jel, Kocak, Istanbul, or Crinone gel® 8 %, Merk Serono, Istanbul) or by 100 mg progesterone injection daily IM (Progynex® ampule, Kocak, Istanbul) until the day of the pregnancy test 12 days after the embryo transfer. Clinical pregnancy is defined as the ultrasound observation of fetal heart movements at 7–8 weeks of gestation.

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) Software, version 17.0. The Levenetest of homogeneity of variances and Kolmogorov–Smirnovtest of normality were performed to choose the appropriate statistical test. Continuous variables would be given as mean±standard deviation if normally distributed, and as median (interquartile range) if not normally distributed. Statistical comparison were be carried out according to the intention to treat by student′s *t*-test, Mann–Whitney *U*-test and χ^2 test for categorical variables, where appropriate. Receiver operating characteristic (ROC) curve analysis were used for statistical analysis. An area under the curve (AUC) value of 0.5 indicated that the test had no discriminative power, and a value of 1.0 indicated that the test had perfect discrimination. Logistic regression analysis was used to assess the association of AMH and other variables with CPR. Differences among groups were considered to be significant if the P value was<0.05.

Results

Table 1 summarizes the clinical and demographic characteristics of the study population. There was no significant difference in terms of BMI, duration of infertility, FSH, LH, E2, TSH, Inhibin-B and prolactin levels between pregnant and non-pregnant women.

The median AMH level of pregnant women was higher than non-pregnant women [3.20 (0.63–9.60) vs 1.15 (0.01–14.90) ng/ml, p<0.001, respectively]. The lowest AMH level in the pregnant group was 0.63 ng/ml. The median AFC and the total number of oocytes were significantly higher in pregnant women compared to non-pregnant women [7 (1–20) vs 5 (0–20), p=0.004; 7 (1–19) vs 4 (0–30), p<0.001, respectively).

There was a significant difference in CPR between the quartiles of AMH and AFC. Cut-off levels of AMH in the 25th and 75th percentiles were 0.63 ng/ml and 2.38 ng/m, respectively. CPR was 1.6 %, 11.9 % and 33.8 % in <25 %, 25 %–75 % and >75 % AMH groups, respectively (p<0.001). CPR was 4.1 %, 16.2 % and 20.6 % in <25 %, 25–75 % and >75 % AFC groups (p=0.041). CPR was 20.6 %, 15.7 % and

Table 1 Comparison of demographical and clinical parameters in pregnant and non-pregnant women

	Pregnant (N:36)	Non-pregnant (N:204)	p
Age, median (min-max)	37.0 (35–41)	38.0 (35–50)	0.005
BMI, mean (kg/m2)	26.80±4.90	26.46±4.56	0.683
Duration ofinfertility (years) median (min-max)	5.5 (1–19)	5.0 (1–23)	0.251
AMH (ng/ml), median (min-max)	3.20 (0.63–9.60)	1.15 (0.01–14.9)	<0.001
FSH (mIU/ml), median (min-max)	5.80 (2.77–14.0)	6.90 (1.25–80.9)	0.069
LH (mIU/ml), median (min-max)	5.0 (1.79–9.90)	4.1 (0.5–82.0)	0.262
E2 (pg/ml)	43.0 (5.25–259.0)	48.0 (7.0–565.0)	0.403
Inhibin-B (pg/mL), mean	79.86±47.32	74.96±65.39	0.803
TSH (mIU/l), mean	2.01±1.14	2.06±1.16	0.793
Prolactin, mean	16.03±6.95	16.58±9.17	0.746
AFC (n), median (min-max)	7 (1–20)	5 (0–20)	0.004
Total Oocyte, median (min-max)	7 (1–19)	4 (0–30)	<0.001
Poor responder n/N (%)	3/36 (8.33)	75/204 (36.76)	<0.001
Cancellation n/N (%)		15/204 (7.35)	

AFC, antral follicle count; AMH, anti-müllerian hormone; BMI, bodymass index; E2, estradiol; FSH, follicle stimulating hormone; LH, luteinizing hormone; TSH, thyroid stimulating hormone. Statistically significant: P<0.05

8.2 % in <25 %, 25–75 % and >75 % FSH groups (p=0.150) (Table 2).

Controlled ovarian stimulation was performed with GnRH antagonist protocol in 110 cycles, long agonist GnRH protocol in 96 cycles and short GnRH agonist protocol in 34 cycles. No differences were observed in CPR between three protocols (16/110 14.5 %, 15/96 15.6 %, 5/34 14.7 %, respectively, p>0.05)

On univariate logistic regression analysis, AMH was an independent predictor of clinical pregnancy rate (OR 1.353; 95 % CI 1.141–1.605; P<0.001). The number of retrieved oocytes, AFC and age were the other independent predictors of clinical pregnant among all the variables studied (Table 3). On multivariate analysis incorporating AMH, the number of retrieved oocytes, AFC, and age, after controlling for the number of retrieved oocytes, AFC and age, AMH remained as a significant predictor of clinical pregnant (OR 1.677; 95 % CI 1.216–2.311; p=0.002; Table 3), with its association strengthened further as indicated by a shift of odds ratio from 1.353 to 1.677.

The ROC curves of the serum AMH concentrations and AFC for the prediction of the clinical pregnancy are depicted in Fig. 1a. The areas under the curves (AUC) were 0.790 (95 % confidence interval [CI], 0.711–0.870) and 0.641 (95 % CI, 0.544–0.737), respectively.

Cut-off level of AMH for the prediction of clinical pregnancy was 1.91 ng/ml. (sensitivity 74 %, specificity 73 %). Poor responders were significantly higher in the non-pregnant group compared to the pregnant group (36.76 % vs 8.33 %, p <0.001). The cut-off value of serum AMH level for the prediction of poor response was 0.97 ng/ml (sensitivity 78.1 %, specificity 73.7 %) (Fig. 1b). AMH levels were significantly correlated with the number of retrieved oocytes (r=0.640, p<0.001) (Fig. 1c).

When the study group was divided according to pregnancy cut-off serum AMH levels (AMH<1.91 ng/ml, AMH≥ 1.91 ng/ml) and ages (Age<38, Age≥38), they showed no statistical difference in CPR between the subgroups (Table 4).

Table 2 Pregnancy rates according to the quartiles of AMH, FSH and AFC

	<25 %		25–75 %		>75 %		
	Range	Pregnancy rate	Range	Pregnancy rate	Range	Pregnancy rate	P
AMH (ng/ml)	≤0.63	1.6 % (1/61)	0.64–2.38	11.9 % (14/117)	≥2.39	%33.8 (21/62)	<0.001
AFC (n)	≤2	4.1 % (2/48)	3–7	16.2 % (21/129)	≥8	20.6 % (13/63)	0.041
FSH (mIU/ml)	≤4.97	20.6 % (12/58)	4.98–9.04	15.7 % (19/121)	≥9.05	8.2 % (5/61)	0.150

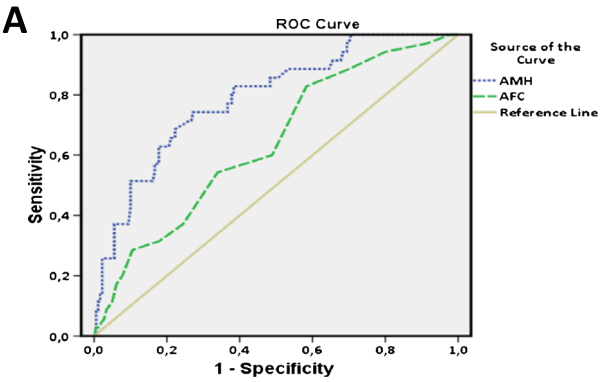
AFC, antral follicle count; AMH, anti-müllerian hormone; FSH, follicle stimulating hormone

Statistically significant: P<0.05

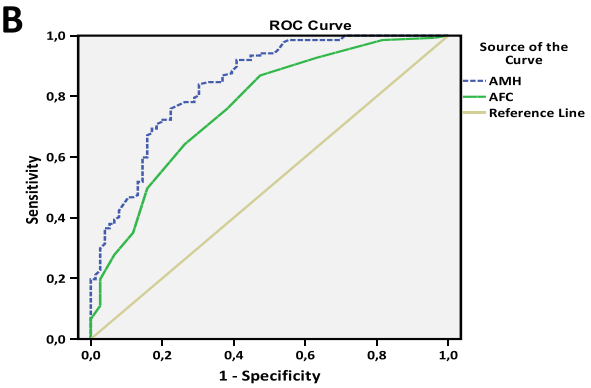
Table 3 Logistic regression analysis of the variables, for the prediction of clinical pregnancy

Parameters	Odds ratio	95 % CI	p
Univariate analysis			
AMH	1.353	1.141–1.605	<0.001
AFC	1.142	1.044–1.249	0.004
The number of retrieved oocytes	1.127	1.051–1.208	0.001
Age	0.788	0.669–0.928	0.004
FSH	0.885	0.780–1.003	0.055
LH	0.984	0.919–1.055	0.657
BMI	1.016	0.942–1.096	0.682
Multivariate analysis of all the significant variables of univariate analysis			
AMH	1.677	1.216–2.311	0.002
AFC	0.944	0.832–1.071	0.369
The number of retrieved oocytes	1.054	0.941–1.181	0.359
Age	0.834	0.684–1.017	0.072

AFC, antral follicle count; AMH, anti-müllerian hormone; BMI, bodymass index; FSH, follicle stimulating hormone; LH, luteinizing hormone. Statistically significant: P<0.05



AMH, AUC 0.790 (95% [CI], 0.711–0.870), cut-off 1.91 ng/ml. (sensitivity 74%, specificity 73%)
AFC, AUC, 0.641 (95% CI, 0.544–0.737)



AMH, AUC 0.843 (95% [CI], 0.787–0.899), cut-off 0.97 ng/ml. (sensitivity78.1%, specificity 73.7%)
AFC, AUC0.763 (95% [CI], 0.696–0.831)

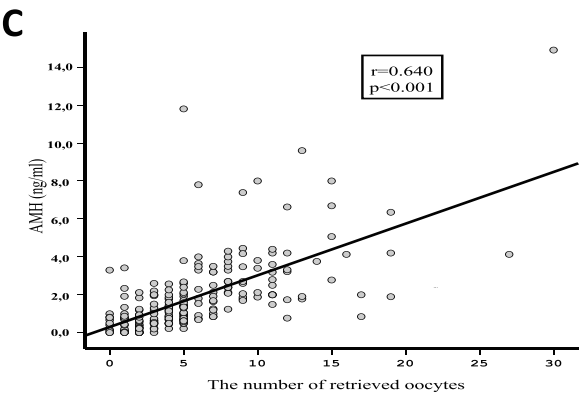


Fig. 1 **a**. The receiver operating characteristic curves for predicting clinical pregnancy. **b**. Receiver operating characteristic curves for AMH and AFC, as predictors of a poor ovarian response to controlled ovarian hyperstimulation.**c**. The relationship between AMH and the number of retrieved oocytes

Discussion

In the current study, we investigated the value of morphometric and basal endocrine parameters during the follicular phases as predictors of IVF outcome in advanced age women (>35 years old). The value of AMH in the prediction of pregnancy has been studied in various studies which showed inconsistent results [14–19].

Broer et al. [20] conducted a meta-analysis of 13 AMH trials and 17 AFC trials. They reported the accuracy for predicting nonpregnancy was poor for both AFC and AMH. There was no significant difference between ROC curves for the prediction of nonpregnancy between both tests (p=0.67). Smeenk et al. [21] found that the AMH level on day 3 of the menstrual cycle was not related to the quality of oocytes and pregnancy rate. In our previous studies, we also demonstrated a non significant correlation between pregnancy and serum AMH values. We stated that under the age of 40; AMH, AFC and FSH cannot predict pregnancy in both normoresponder and PCOS patient groups in IVF cycles. We also didn't detect a significant difference in clinical pregnancy rates between the quartiles of AMH, FSH and AFC. The clinical pregnancy rate was 21 % in patients that had serum AMH levels lower than 1.81 ng/ml (<25%percentile). The pregnancy rate increased slightly with the concomitant increase of serum AMH but the mean serum AMH values for the pregnant and nonpregnant groups were nonsignificant [18, 19]. In these our published studies, there were no significant difference in AMH levels between pregnant and non-pregnant under aged 40 years women in both normoresponder and PCOS patient groups (3.9±2.5 vs 3.8±3.0 ng/ml, p=0.831; 6.79±2.9 vs 7.16±4.29 ng/ml, p=0.594, respectively). However, in the current study, we found that there was significant difference in AMH levels between pregnant and nonpregnant advanced age

Table 4 Pregnancy rates according to age and pregnancy cut-off levels

	AMH<1.91			AMH≥1,91		
	Age 35–38 (N=58)	Age≥38 (N=95)	p	Age 35–38 (N=51)	Age≥38 (N=36)	p
Pregnancy (+), n, (%)	4 (6.9)	5 (5.3)	0.676	17 (33.3)	10 (27.8)	0.581
Pregnancy (-), n, (%)	54 (93.1)	90 (94.7)		34 (66.6)	26 (72.2)	

AMH, anti-müllerian hormone. Statistically significant: P<0.05

women [median 3.20 (0.63–9.60) vs 1.15 (0.01–14.90) ng/ml, p<0.001, respectively]. AMH does seem clinically useful for predicting pregnancy in advanced age women.

Friden et al. evaluated 127 women with a median age of 42 years (range 39–46) and demonstrated that women with a serum AMH above 8.6 pmol/L had a good chance of achieving live birth after IVF/ ICSI treatment and concluded that AMH is useful for identifying a good prognosis group in women of advanced reproductive age [22]. Choi et al. evaluated 370 women (20–42 years old) and demonstrated that statistical differences were found in the number of oocytes retrieved and clinical pregnancy rates [23]. Wang et al. demonstrated that women between 34 and 41 years old with higher serum AMH concentrations are associated with significantly greater chances of pregnancy (p<0.01) [14]. In a large prospective study included 340 patients, Nelson et al. demonstrated that a single measurement of circulating AMH can be used to individualize treatment strategies for IVF [24].

In the present study, we evaluated the clinical pregnancy rates according to the quartiles of AMH. We observed that clinical pregnancy rates tended to increase with increasing quartiles of serum AMH. The pregnancy rate was 1.6 % in the patients whose serum AMH level was lower than 0.63 ng/ml and 33.8 % in patients whose serum AMH level was higher than 2.39 ng/ml. The lowest level of serum AMH was 0.63 ng/ml in the pregnant group. Wang et al [14] investigated the relationship between IVF clinical pregnancy rates per initiated cycle and serum AMH tertiles stratified by age in 1,558 women in all age groups and determined that age influenced the AMH & clinical pregnancy rate relationship. They showed that for women aged ≥42 years with AMH ≤0.29 ng/ml, CPR was significantly lower than those of the middle and higher quartiles, whereas CPR for women in the middle and highest tertiles were not significantly different.

In the present study, on logistic regression analysis, AMH was an independent predictor of clinical pregnancy rate (OR 1.353; 95 % CI 1.141–1.605; P<0.001). After controlling for the other independent variables (the number of retrieved oocytes, AFC and age), the significant association between AMH and clinical pregnancy rate remained strong (OR 1.677; 95 % CI 1.216–2.311; p=0.002) on multivariate logistic regression analysis. Similarly, in a recently published meta-analysis, Iliodromiti et al concluded that AMH adds some value in predicting live birth in women undergoing assisted conception, and this is independent of age or AMH assay. However they stated that its predictive accuracy is poor and should not be over-interpreted [25].

Al-Inany et al. conducted a meta-analysis of 45 RCTs comparing long agonist versus antagonist protocols in women undergoing IVF or ICSI. They concluded that there was no evidence of a statistically significant difference in rates of live-births (9 RCTs; odds ratio (OR) 0.86, 95 % CI 0.69 to 1.08) [26]. Similarly, in the present study, no differences were observed in CPR between three protocols (16/110 14.5 %, 15/96 15.6 %, 5/34 14.7 %, respectively, $p>0.05$)

In conclusion, AMH is an effective measure of quantitative ovarian reserve and it can predict ovarian response to controlled stimulation for advanced age women. The clinical pregnancy rate tends to increase as AMH increases. The AMH levels are more useful than FSH and AFC for the prediction of poor response, pregnancy and the number of retrieved ocytes in women who underwent IVF cycles at the age of 35 and older.

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