Relevance of anti-Müllerian hormone on in vitro fertilization outcome

E. Celik, E. Bastu, O. Dural, C. Yasa, F. Buyru

Department of Obstetrics and Gynecology, Istanbul University School of Medicine, Istanbul (Turkey)

Summary

Purpose: The aim of this study was to investigate the relevance of serum and follicular anti-Müllerian hormone (AMH) concentrations on ovarian reserve and clinical pregnancy. *Materials and Methods:* Thirty patients were prospectively included in this study. Serum AMH levels were quantitatively measured on the follicle aspiration day. Retrieving less than five oocytes was defined as *poor response*. Eleven days after embryo transfer, beta-human chorionic gonadotropin (β-hCG) level in the blood was measured. Two weeks after the β-hCG test, a clinical pregnancy was confirmed by transvaginal ultrasound (TVUS). *Results:* There was a statistically significant correlation between serum AMH and number of retrieved oocytes ($p = 0.024$). There was a correlation between the number of retrieved oocytes and baseline antral follicle count (AFC), between ovarian reserve and baseline follicle-stimulating hormone (FSH), and between ovarian reserve and serum AMH ($p < 0.05$). Serum AMH cut-off value for the normal ovarian reserve was calculated as 0.37ng/ml (sensitivity 71.43%, specificity 66.67%, positive prediction 83.33%, negative prediction 50%). *Conclusion:* Increasing use of serum AMH will be of considerable benefit. Consequently, the observed positive correlation between serum AMH and ovarian reserve will require larger sampling to refine the role of AMH.

Key words: Anti-Müllerian hormone; Follicular anti-Müllerian hormone; In vitro fertilization; Serum anti-Müllerian hormone.

Introduction

Socio-economic changes in several societies have an impact on couples' desires to have children. Consequently, in the last decade, more couples postpone their plans to have children. On the other hand, it has been well-established that with an increasing age, female fertility decreases. This has been clearly demonstrated by the age-dependent success rates of assisted reproductive technology (ART) therapy [1]. Changes in ovarian reserve, which are defined as the number and quality of the follicles and ooctyes in the ovaries at a given age, lead to age-related female infertility [2]. For ovarian reserve testing prior to ART, the age of the patient remains the first line of choice as a predictor. However, a test that can provide accurate information on a patient's ovarian reserve would be of immense help to any clinician.

Recently, a role for anti-Müllerian hormone (AMH) in ovarian function has become apparent by the aid of animal studies [3]. The release of AMH from ovarian granulosa cells paves the way to measurable serum levels, which are proportional to the number of follicles in the ovaries. Hence, AMH can be considered as one of the markers for ovarian aging, since the number of follicles decreases with age [4].

The aim of this study was to investigate the relevance of serum and follicular anti-Müllerian hormone (AMH) concentrations on ovarian reserve and clinical pregnancy, which are important success parameters in an ART therapy like *in vitro* fertilization (IVF).

Revised manuscript accepted for publication May 18, 2012

Materials and Methods

Thirty IVF patients in the Infertility Clinic of Istanbul University School of Medicine were prospectively included within this study. Inclusion criteria were as follows: 18-45 years of age, no history of an endocrine disorder of the thyroid, adrenals, etc., and no history of an ovary-related surgery. Approval of the ethics committee and informed consent from all participants were obtained prior to the treatment.

Patients were assessed by day-3 hormone profile follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), progesterone (P), prolactin (PRL), thyroid-stimulating hormone (TSH), and hysterosalpingography. Transvaginal ultrasonography (TVUS) was performed on the second or third day of the menstrual cycle and the size of the uterus and of the ovaries, follicle count and diameter measurements were recorded. Serum AMH levels were quantitatively measured on the follicle aspiration day in the microbiology laboratory of Istanbul University School of Medicine by enzyme-linked immunosorbent assay (ELISA) and applied uniformly for all patient samples. Intra- and interassay coefficients of variation with serum controlled samples were 4.6% and 5.2%, respectively. The type of gonadotropin releasing hormone (GnRH) agonist long protocol or GnRH antagonist protocol for ovarian stimulation was determined by the patient's doctor, based on her age, and the status of ovarian reserve markers. The number of follicles and endometrial thickness were recorded at each follow-up.

Follicular fluid sample of the leading follicle collected during oocyte retrieval (OCT) was centrifuged at 3,000 cycles/min and stored at -80°C for follicular AMH measurement. All follicles that were ≥ 14 mm in size were aspirated. The number of retrieved oocytes was recorded. Retrieving less than five oocytes was defined as a *poor response*. Three days after retrieval, one to three embryos (grade-1) were transferred to the uterine cavity depending on the age of the patient. Eleven days after embryo transfer, beta-chorionic gonadotropin (β-hCG) level in the blood was measured. If β-hCG level was > 5 mIU/ml in either measurement, it was considered *positive* β-hCG and patients with such levels were regarded as biochemically pregnant. Two

weeks after the β-hCG test, clinical pregnancy was confirmed by TVUS.

All statistical calculations were performed using the NCSS 2007 and PASS 2008 (NCSS, Kaysville, UT, USA). Data are presented as mean ± SD. Parametric variables were evaluated by Student's t-test, while non-parametric variables were evaluated by the Mann-Whitney U test. Receiver operating characteristic (ROC) curve analysis was used to determine cut-off values for those parameters significantly associated with clinical pregnancy and ovarian reserve. Statistical significance was defined as $p < 0.05$.

Results

Demographic and cycle characteristics of the study population are presented in Table 1. There was no correlation between serum AMH and age, baseline FSH or baseline antral follicle count (AFC) ($p > 0.05$). On the other hand, there was a statistically significant correlation between serum AMH and number of retrieved oocytes (p = 0.024). There was no correlation between follicular AMH and age, number of retrieved oocytes, baseline FSH or baseline AFC ($p > 0.05$). There was a negative correlation between baseline FSH and number of retrieved oocytes ($p < 0.01$) and also between baseline FSH and baseline AFC ($p < 0.05$). There was a correlation between the number of retrieved oocytes and baseline AFC ($p < 0.05$). There was no correlation between age and baseline FSH, number of retrieved ooctyes, or baseline AFC ($p > 0.05$). There was no correlation between clinical pregnancy and serum/follicular AMH, age, baseline AFC, baseline FSH, baseline E2, period of infertility, number of metaphase II ooctyes, number of retrieved oocytes, or endometrial thickness ($p > 0.05$).

The area under the ROC curve for predicting clinical pregnancy was larger for follicular AMH (0.630; 95% (confidence interval) CI: 0.419-0.841; p = 0.28) than for serum AMH (0.594; 95% CI: 0.344-0.842; p = 0.43), age (0.446; 95% CI: 0.221-0.670; p = 0.65) and FSH (0.383; 95% CI: 0.143-0.623; p = 0.33), but not statistically significant.

There was no correlation between ovarian reserve and follicular AMH, age, baseline AFC, baseline E2, or period of infertility ($p > 0.05$). There was a correlation between ovarian reserve and baseline FSH ($p < 0.05$). Baseline FSH of patients with a normal ovarian reserve was significantly lower than patients with a poor ovarian reserve (5.95 ± 1.85 vs 9.02 ± 4.52). There was a correlation between ovarian reserve and serum AMH ($p < 0.05$). Serum AMH concentrations of patients with a normal ovarian reserve were significantly higher than patients with a poor ovarian reserve (0.73 ± 0.63 vs 0.28 ± 0.19).

The area under the ROC curve for poor ovarian responders was larger for serum AMH (0.775; 95% CI: 0.604-0.946; p = 0.19) than for AFC (0.714; 95% CI: 0.518-0.911; p = 0.067) and follicular AMH (0.545; 95% CI: 0.313-0.777; p = 0.700) and statistically significant. Due to the correlation between serum AMH and ovarian reserve, ROC curve analysis was used to determine the cut-off value for serum AMH significantly associated with ovarian reserve. Serum AMH cut-off value for a normal ovarian reserve (five oocytes or more) was calculated as 0.37 ng/ml (sensitivity 71.43%, specificity 66.67%, positive prediction 83.33%, negative prediction 50%) (Table 2).

Table 1. — *Patient characteristics (p = 30).*

	Min - Max	Mean ± SD
Age (years)	24 - 43	31.80 ± 4.67
Period of infertility (years)	1 - 20	5.30 ± 4.71
AFC	2 - 16	9.23 ± 3.11 (10)
Baseline FSH (mIU/ml)	3.2 - 19.4	6.87 ± 3.17 (6.35)
Baseline E2 (pg/ml)	19.2 - 138.0	46.07 ± 24.46 (39.55)

Table 2. — *Serum AMH cut-off values.*

Serum AMH (ng/ml)	Sensitivity (%)	Specificity (%)	Positive prediction (%)	Negative prediction (%)
0.25	85.71	44.44	78.26	57.14
0.30	76.19	55.56	80.00	50.00
0.37	*71.43*	*66.67*	*83.33*	*50.00*
0.40	61.90	77.78	86.67	46.67
0.45	52.38	77.78	84.62	41.18
0.50	47.62	88.89	90.91	42.11
0.60	47.62	100.00	100.00	45.00
0.70	38.10	100.00	100.00	40.91

Table 3. — *Patient characteristics as a function of serum AMH.*

Serum AMH	AMH ≤ 0.37 (ng/ml) Mean ± SD	AMH > 0.37 (ng/ml) Mean ± SD	[a]*p*
[a]Age (years)	32.11 ± 5.69	31.33 ± 2.67	0.663
Baseline FSH (mIU/ml)	8.07 ± 4.00 (7.05)	6.07 ± 2.25 (5.75)	0.046*
AFC	7.83±3.12 (8)	10.17 ± 2.81 (10)	0.048*
Number of metaphase II ooctyes	7.17 ± 6.93	9.50 ± 5.14	0.078
Number of retrieved ooctyes	8.83 ± 7.76 (5.5)	12.22 ± 6.60 (10.5)	0.042*

[a]Student t test; [a]Mann Whitney U test; *$p < 0.05$.

Table 3 presents that patients with serum AMH ≤ 0.37 ng/ml demonstrated a higher baseline FSH ($p < 0.05$), had a lower baseline AFC ($p < 0.05$), had fewer numbers of retrieved ooctyes ($p < 0.05$), and had fewer numbers of metaphase II oocytes, but was not statistically significant (7.17 ± 6.93 vs 9.50 ± 5.14; p = 0.078). Interestingly, age was not a statistically significant factor (32.11 ± 5.69 vs 31.33 ± 2.67; p = 0.663)

Discussion

The correct assessment of ovarian reserve is crucial for a successful IVF outcome. In this study, the authors sought to determine whether serum or follicular AMH would have any use in predicting ovarian reserve and subsequently, clinical pregnancy rates.

Mattukrisha *et al*. found a correlation between serum AMH and number of retrieved ooctyes in a study consisting of 69 patients ($p < 0.001$) [5]. Buyuk *et al*. carried out a somewhat similar study and found that serum AMH levels strongly correlate with the number of retrieved

Table 4. — *Serum AMH cut-off values in various studies*.

Studies	Serum AMH (ng/ml)	Sensitivity	Specificity
Muttukrisha *et al*. [4]	0.10	0.76	0.88
Penarrubia *et al*. [16]	0.69	0.40	0.92
Ebner *et al*. [17]	1.66	0.69	0.86
Tremellen *et al*. [18]	1.13	0.80	0.85
McIlveen *et al*. [19]	1.25	0.58	0.75
Freour *et al*. [20]	1.30	0.44	1.00
Smeenk *et al*. [21]	1.40	0.62	0.73
Nakhuda *et al*. [22]	0.35	0.91	0.82
Nelson *et al*. [23]	0.14	0.38	0.99
Barad *et al*. [24]	0.50	0.87	0.84
Riggs *et al*. [7]	0.83	0.82	0.79
Gnoth *et al*. [25]	1.26	0.97	0.41
Nardo *et al*. [15]	1.00	0.87	0.67
Jayaprakasan *et al*. [13]	0.99	1.00	0.73
Buyuk *et al*. [6]	0.60	0.70	0.70
Tolikas *et al*. [12]	2.74	0.69	0.70

oocytes as well ($p < 0.0001$) [6]. The findings in the presented study yielded a correlation between serum AMH and the number of retrieved ooctyes as well (p = 0.024). Sills *et al*. [7], in a study of 79 patients, found a moderate positive correlation between serum AMH and the number of metaphase II oocytes; the present study also revealed a correlation that was not statistically significant (p = 0.078). Riggs *et al*. also stated that serum AMH values correlated the best with the number of retrieved oocytes (p = 0.001) relative to age ($p < 0.01$) and FSH ($p < 0.01$) [8]. In the present study, there was no correlation between serum AMH and age (p = 0.929) or baseline FSH (p = 0.111).

The authors demonstrated a clinical pregnancy rate of 27% while none of the parameters achieved a statistical significance in predicting the clinical outcome. In contrast, Wu *et al*. conducted a study that included 60 patients and found a statistically significant correlation between clinical pregnancy outcome and day-3 AMH ($p < 0.05$) [9]. On the other hand, Buyuk *et al*. found a correlation between serum AMH and the clinical outcome that was not statistically significant (p = 0.1) [6]. Sills *et al*. also indicated a higher serum AMH level in patients who attained a clinical pregnancy, but the difference was not significant (p = 0.14) [7]. Additionally, a meta-analysis by Broekmans *et al*. revealed that the accuracy of the current ovarian reserve tests, including serum AMH, for predicting the occurrence of pregnancy, is very limited [10].

Poor ovarian reserve had a strong correlation with serum AMH in the present study, which was seen in 30% of the sample size (p = 0.017). Van Rooij *et al*. defined "a poor response" as retrieving less than four oocytes and found it strongly correlated with serum AMH as well ($p < 0.01$) [11]. In a recent study by Tolikas *et al*., "a poor response" was again defined as retrieving less than four oocytes [12]. These researchers presented significant differences between poor and normal responders regarding FSH (p = 0.019) and serum AMH (p = 0.002), but not for follicular AMH (p = 0.183). The present study also demonstrated significant differences between poor and normal responders regarding FSH (p = 0.01), while no difference was found regarding follicular AMH (p = 0.722).

The strong correlation between serum AMH and ovarian reserve led the authors to determine the cut-off value for serum AMH by using ROC curve. Serum AMH cut-off value for the normal ovarian reserve (five oocytes or more) was calculated as 0.37 ng/ml. This data suggests that women with increased serum AMH concentrations above 0.37 ng/ml may be regarded as a better-prognosis group during IVF cycles than women with a serum AMH below 0.37 ng/ml. There have been several studies that attempted to determine a cut-off serum AMH value for a normal ovarian reserve, albeit with varying results (Table 4). In more recent ones, Jayakprakasan *et al*. [13] found 0.99 ng/ml as the optimum serum AMH cut-off value, Buyuk *et al*. [6] argued that women who had a serum AMH level of 0.6 ng/ml or higher had a better ovarian reserve, while Tolikas *et al*. presented a higher serum AMH cut-off value of 2.74 ng/ml [12]. In a review, Broer *et al*. concluded that serum AMH was able to predict ovarian reserve but could not predict pregnancy after ART treatment [14]. Therefore, this review's authors advocated the determination of a low serum AMH cut-off value due to the importance of statistical specificity in poor ovarian reserve.

The findings in this study indicated that after serum AMH, baseline AFC was the most effective parameter in predicting ovarian reserve, yet it was not statistically significant (p = 0.067). On the other hand, the patients with serum AMH ≤ 0.37 ng/ml had a significantly lower baseline AFC ($p < 0.05$). Both Jayaprakasan *et al*. [13] and Tolikas *et al*. [12] concluded that a baseline AFC and serum AMH are significant predictors of poor ovarian reserve to ovarian stimulation during IVF, while Nardo *et al*. concluded that serum AMH was superior to AFC in its ability to predict poor response [15].

In conclusion, serum AMH appears to represent a compelling tool for the assessment of ovarian reserve, yet it yields a non-significant predictive value for clinical pregnancy. While bearing the caveats, it is still clear that the increasing use of serum AMH will be of considerable benefit in reproductive medicine. Consequently, the observed positive correlation between serum AMH and ovarian reserve will require larger sampling to refine the role of AMH in IVF strategies.

References

[1] Nyboe A.A., Goossens V., Bhattacharya S., Ferraretti A.P., Kupka M.S., de Mouon J., Nygren K.G., European IVF-monitoring (EIM) Consortium, for the European Society of Human Reproduction and Embryology (ESHRE): "Assisted reproductive technology and intrauterine inseminations in Europe, 2005: results generated from European registers by ESHRE - ESHRE. The European IVF Monitoring Programme (EIM), for the European Society of Human Reproduction and Embryology (ESHRE)". *Hum. Reprod.,* 2009, *24,* 1267.

[2] Stephen E.H., Chandra A.: "Declining estimates of infertility in the United States: 1982-2002". *Fertil. Steril.,* 2006, *86,* 516.
[3] Visser J.A., Themmen A.P.: "Anti-Mullerian hormone and folliculogenesis". *Mol. Cell. Endocrinol.,* 2005, *234*, 81.
[4] Kevenaar M.E., Meerasahib M.F., Kramer P., van de Lang-Born B.M., de Jong F.H., Groome N.P. *et al.*: "Serum antimullerian hormone levels reflect the size of the primordial follicle pool in mice". *Endocrinology,* 2006, *147,* 3228.
[5] Muttukrishna S., Suharjono H., McGarrigle H., Sathanandan M.: "Inhibin B and anti-Müllerian hormone: markers of ovarian reserve in IVF/ICSI patients?". *BJOG,* 2004, *111,* 1248.
[6] Buyuk E., Seifer D.B., Younger J., Grazi R.V., Lieman H.: "Random anti-Müllerian hormone (AMH) is a predictor of ovarian reserve in women with elevated baseline early follicular follicle-stimulation hormone levels". *Fertil. Steril.,* 2011, *95,* 2369.
[7] Sills E.S., Collins G.S., Brady A.C., Walsh D.J., Marron K.D., Peck A.C. *et al.*: "Bivariate analysis of basal serum anti-Müllerian hormone measurements and human blastocyst development after IVF". *Reprod. Biol. Endocrinol.,* 2011, *9,* 153.
[8] Riggs R.M., Duran E.H., Baker M.W., Kimble T.D., Hobeika E., Yin L. *et al.*: "Assessment of ovarian reserve with anti-Müllerian hormone: a comparison of the predictive value of anti-Müllerian hormone, follicle-stimulating hormone, inhibin B, and age". *Am. J. Obstet. Gynecol.,* 2008, *199*, 202.
[9] Wu C.H., Chen Y.C., Wu H.H., Yang J.G., Chang Y.J., Tsai H.D.: "Serum anti-Müllerian hormone predicts ovarian reserve and cycle outcome in IVF patients". *J. Assist. Reprod. Genet.,* 2009, *26,* 383.
[10] Broekmans F.J., Kwee J., Hendriks D.J., Mol B.W., Lambalk C.B.: "A systematic review of tests predicting ovarian reserve and IVF outcome". *Hum. Reprod. Update,* 2006, *12,* 685.
[11] van Rooij I.A., Broekmans F.J., te Velde E.R., Fauser B.C., Bancsi L.F., de Jong F.H., Themmen A.P.: "Serum anti-Müllerian hormone levels: a novel measure of ovarian reserve". *Hum. Reprod.,* 2002, *17,* 3065.
[12] Tolikas A., Tsakos E., Gerou S., Prapas Y., Loufopoulos A.: "Anti-Müllerian Hormone (AMH) levels in serum and follicular fluid as predictors of ovarian reserve in stimulated (IVF and ICSI) cycles". *Hum. Fertil.,* 2011, *14*, 246.
[13] Jayaprakasan K., Deb S., Batcha M., Hopkisson J., Johnson I., Campbell B., Raine-Fenning N.: "The cohort of antral follicles measuring 2-6mm reflects the quantitative status of ovarian reserve as assessed by serum levels of anti-Müllerian hormone and response to controlled ovarian stimulation". *Fertil. Steril.,* 2010, *94,* 1775.
[14] Broer S.L., Mol B., Dólleman M., Fauser B.C., Broekmans F.J.: "The role of anti-Müllerian hormone assessment in assisted reproductive technology outcome". *Curr. Opin. Obstet. Gynecol.,* 2010, *22,* 193.
[15] Nardo L.G., Gelbaya T.A., Wilkinson H., Roberts S.A., Yates A., Pemberton P., Laing I.: "Circulating basal anti-Müllerian hormone levels as predictor of ovarian reserve in women undergoing ovarian stimulation for in vitro fertilization". *Fertil. Steril.,* 2009, *92,* 1586.
[16] Peñarrubia J., Fábregues F., Manau D., Creus M., Casals G., Casamaitjana R. *et al.*: "Basal and stimulation day 5 anti-Müllerian hormone serum concentrations as predictors of ovarian reserve and pregnancy in assisted reproductive technology cycles stimulated with gonadotropin-releasing hormone agonist-gonadotropin treatment". *Hum. Reprod.,* 2005, *20*, 915.
[17] Ebner T., Sommergruber M., Moser M., Shebl O., Schreier-Lechner E., Tews G.: "Basal level of anti-Müllerian hormone is associated with oocyte quality in stimulated cycles". *Hum. Reprod.,* 2006, *21*, 2022.
[18] Tremellen K.P., Kolo M., Gilmore A., Lekamge D.N.: "Anti-Müllerian hormone as a marker of ovarian reserve". *Aust. N.Z.J. Obstet. Gynaecol.,* 2005*, 45*, 20.
[19] McIlveen M., Skull J.D., Ledger W.L.: "Evaluation of the utility of multiple endocrine and ultrasound measures of ovarian reserve in the prediction of cycle cancellation in a high-risk IVF population". *Hum. Reprod.,* 2007, *22,* 778.
[20] Fréour T., Mirallié S., Bach-Ngohou K., Denis M., Barrière P., Masson D.: "Measurement of serum anti-Müllerian hormone by Beckman Coulter ELISA and DSL ELISA: comparison and relevance in assisted reproduction technology (ART)". *Clin. Chim. Acta,* 2007, *375,* 162.
[21] Smeenk J.M., Sweep F.C., Zielhuis G.A., Kremer J.A., Thomas C.M., Braat D.D.: "Antimüllerian hormone predicts ovarian responsiveness, but not embryo quality or pregnancy, after in vitro fertilization or intracytoplasmic sperm injection". *Fertil. Steril.,* 2007, *87,* 223.
[22] Nakhuda G.S., Sauer M.V., Wang J.G., Ferin M., Lobo R.A.: "Müllerian inhibiting substance is an accurate marker of ovarian reserve in women of advanced reproductive age undergoing IVF". *Reprod. Biomed. Online,* 2007, *14,* 450.
[23] Nelson S.M., Yates R.W., Fleming R.: "Serum anti-Müllerian hormone and FSH: prediction of live birth and extremes of response in stimulated cycles-implications for individualization of therapy". *Hum. Reprod.,* 2007, *22*, 2414.
[24] Barad D.H., Weghofer A., Gleicher N.: "Comparing anti-Müllerian hormone (AMH) and follicle-stimulating hormone (FSH) as predictors of ovarian function". *Fertil. Steril.,* 2009, *91,* 1553.
[25] Gnoth C., Schuring A.N., Friol K., Tigges J., Mallmann P., Godehardt E.: "Relevance of anti-Müllerian hormone measurement in a routine IVF program". *Hum. Reprod.,* 2008, *23*, 1359.

Address reprint requests to:
E. BASTU, M.D.
Istanbul University School of Medicine
Department of Obstetrics and Gynecology
Division of Infertility
Capa 34093, Istanbul (Turkey)
e-mail: ercan.bastu@istanbul.edu.tr