

The correlation of the antral follicle count and Serum anti-mullerian hormone

Serum anti-mülleryan hormon ve antral folikül sayısı ilişkisi

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Abstract

Objective: To compare the value of the basal serum anti-Müllerian hormone (AMH) level with most of the established ovarian reserve tests.

Material and Methods: A total of 141 infertile women was studied prospectively. On cycle day 3, serum levels of AMH, inhibin B, estradiol (E₂), FSH and LH levels were measured, and the number of early antral follicles (2-6 mm in diameter) estimated at ultrasound scanning to compare the strengths of hormonal-follicular correlations.

Results: The mean age of the participants was 29.18±5.54. The mean AMH and total AFC on day 3 were 2.23±1.90 ng/ml and 8.35±2.83, respectively. Serum AMH levels were more tightly correlated ($p<0.001$) with number of the early antral follicle count ($r=0.467$, $p<0.0001$) than age and serum levels of FSH ($r=-0.400$, $p<0.001$; $r=-0.299$, $p<0.001$ respectively). No correlation was detected between serum levels of inhibin B, E₂, and LH ($r=0.154$, $p=0.06$; $p=0.31$; $r=-0.085$ and $r=0.067$, $p=0.42$) and AFC.

Conclusion: Serum AMH levels showed a strong correlation with AFC, and also this correlation is stronger than the other ovarian reserve parameters. (J Turkish-German Gynecol Assoc 2010; 11: 212-5)

Key words: Anti-mullerian hormone; ovarian reserve; antral follicle count

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Özet

Amaç: Bu çalışmada basal serum bazal anti-Müllerian hormone (AMH) seviyesinin diğer sık kullanılan over rezerv testleri ile karşılaştırılması amaçlanmıştır.

Gereç ve Yöntemler: Toplam 141 infertil kadın çalışmaya alındı. Üçüncü gün serum AMH, inhibin B, östradiol (E₂), FSH ve LH seviyeleri ölçümü ve ultrasonografik olarak erken antral folikül (2-6 mm çaplı) sayımı yapılarak hormonal-foliküler korelasyon değerlendirildi.

Bulgular: Olguların ortalama yaşı 29.18±5.54 idi. Ortalama AMH ve total AFC sırasıyla 2.23±1.90 ng/ml ve 8.35±2.83 olarak saptandı. Serum AMH seviyesi ile erken antral folikül sayısı arasındaki korelasyon ($r=0.467$, $p<0.0001$), yaş ve serum FSH düzeyleri ile erken antral folikül sayısı arasındaki korelasyondan (sırasıyla; $r=-0.400$, $p<0.001$; $r=0.299$, $p<0.001$) daha güçlü idi ($p<0.001$). Serum inhibin B, E₂, ve LH seviyeleri ile AFC arasında korelasyon saptanmadı ($r=0.154$, $p=0.06$; $p=0.31$; $r=-0.085$ and $r=0.067$, $p=0.42$).

Sonuç: Serum AMH seviyesi, AFC ile sıkı bir biçimde ilişkilidir ve aynı zamanda bu ilişki diğer over rezervi değerlendirme parametrelerinden daha güçlüdür. (J Turkish-German Gynecol Assoc 2010; 11: 212-5)

Anahtar kelimeler: Anti-mülleryan hormon; over rezervi; antral folikül sayısı

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Introduction

Ovarian reserve is described as the quantity of the ovarian follicular cohort and quality of the oocytes (1). The assessment of the ovarian reserve needs for identification of the response of controlled ovarian stimulation (COH). This assessment facilitates appropriate pretreatment counseling and modification of an individual's treatment protocol in an attempt to maximize their potential response. Assessing an individual's ovarian reserve includes age, estradiol (E₂) and basal follicle-stimulating hormone (FSH) levels. Antral follicle count, serum inhibin B levels, ovarian volume, and vascular resistance have also been studied as markers of ovarian reserve. The antral follicle count (AFC) have been widely used as the ovarian reserve test, due to convenience of the ultrasonographic tools usage. Follicle counts can be performed easily with the help of the high quality resolution of the sonographic systems (2-10). Although there are well-known difficulties to obtain correct AFC such as high inter-observer differences and ana-

tomical variations (3), it has been suggested that the ability of AFC to predict poor response might be significantly better than basal FSH. Thus, AFC has been considered the "test of first choice" by some investigators (2, 11).

Recently; serum anti-Müllerian hormone (AMH) has been commonly studied as a potential new test for ovarian reserve. AMH, also known as Müllerian-inhibiting substance, is a dimeric glycoprotein that belongs to the transforming growth factor- β family (12-15). Antimüllerian hormone is secreted by small antral follicles and in reproductive-aged women and it is expressed by granulosa cells of the ovary (16). In the ovary, AMH inhibits initial primordial follicles recruitment and decreases the sensitivity of preantral and small antral follicles to FSH and hence suggesting its role in intrafollicular and interfollicular coordination of follicle development (17, 18). Recently it has been shown that the higher AMH levels were associated with the greater numbers of retrieved oocytes and improved embryo morphology in the IVF cycles (10, 15, 19, 20). There has been a controversy between the correlation of

the AFC and the other ovarian reserve tests such as age, AMH, basal FSH, E₂ and inhibin-B. Therefore, the aim of this study is to investigate the correlation between AFC and age, AMH, basal FSH, Estradiol (E₂), LH and inhibin-B levels in a selected population of women who were referred for the fertility treatment.

Materials and Methods

A total of 141 patients who were evaluated prior to their first treatment cycle were prospectively included into our study based on the following criteria: regular menstrual cycles (21-35 days), presence of both ovaries, age less than 45 years. Subjects were excluded if they had abnormal uterine bleeding, evidence of endocrine disorders (normal thyroid stimulating hormone, prolactin, testosterone and androstenedione), suboptimal visualization of the ovary by transvaginal ultrasonography, an ovarian cyst or follicle measuring 20 mm or more in diameter and a history of ovarian surgery. The Institutional Review Board approval and written informed consent were achieved for this study. On the third day of the spontaneous cycle, all patients had a transvaginal scan by the same investigator (N.I) using a GE General logiq 400 pro (GE medical systems, Korea CO., LTD. Sungdam Shi, KS) 5MHZ ultrasound probe to assess the number of antral follicles, measuring 2-6 mm, as described previously (3). Each ovary was measured in three planes and ovarian volume was calculated using the prolate ellipsoid formula (V=D1xD2xD3x0.523). D1, D2 and D3 are being the three maximal longitudinal, antero-posterior and transverse diameters, respectively (21). On the same day, a venous blood sample was obtained for the measurement of AMH, FSH, LH, E₂ and inhibin B.

Measurement of serum AMH levels was performed using the MIS/AMH enzyme-linked immunosorbent assay kit DSL (diagnostic systems laboratories, Inc./USA). Inhibin B was measured using the Inhibin B enzyme-linked immunosorbent assay kit (Diagnostic System Lab, Inc./USA). FSH, LH and E₂ levels were assessed in plasma with the AxSYM immunoanalyser (Abbott Laboratories, Abbott Park, IL, USA).

Statistical analysis was performed using SPSS (version 13.0; SPSS, Inc., Chicago, IL). The data was expressed by means and the standard deviations. Relationship between two different continuous variables was assessed by Pearson Correlation. The Fisher r to z-test was used to determine if the coefficient of correlation (r) was significantly different from zero. A p<0.05 was considered as statistically significant.

Results

The mean age of the participants was 29.18±5.54 (range 23-44) and 69.5% (n=98) of the patients had primary infertility. The mean AMH and total AFC on day 3 were 2.23±1.90 and 8.35±2.83, respectively. Table 1 summarizes age, BMI mean of the FSH, LH, E₂, AMH, inhibin B and total AFC and the mean ovarian volume of the participants.

Correlations of the number of antral follicles, the mean ovarian volume, AMH and the others ovarian reserve parameters are shown in Table 2. Unlike, inhibin B, serum levels of E₂ and LH (r=0.154, p=0.06; p=0.31; r=-0.085 and r=0.067, p=0.42), those of age, AMH, and FSH were significantly correlated with the number of early antral follicles on cycle day 3.

It is noteworthy that the correlation between number of early antral follicles and serum AMH levels (r=0.467, p<0.0001) was significantly stronger (p<0.0001) than age and serum levels of FSH (r=-0.400, p<0.001; r=-0.299, p<0.001 respectively). In addition to this, serum AMH levels showed a stronger correlation (p<0.001) with the mean ovarian volume (r=0.373, p<0.0001) than did those of age (r=-0.182, p=0.03), inhibin B (r=0.180, p=0.03), E₂ (r=0.079, p=NS), FSH (r=-0.276, p=0.001) and LH (r=-0.005, p=NS). Incidentally, serum AMH levels were significantly correlated with those of age (r=-0.182, p=0.03), inhibin B (r=0.259, p=0.002) and FSH (r=-0.290, p<0.001), but not with those of E₂ and LH.

Table 1. The demographics and FSH, LH, E₂, AMH, Inhibin B, and AFC and the mean ovarian volume on day 3 of the participants

n=141	Mean	±SD
Age (years)	29.18	5.54
BMI (kg/m ²)	24.76	3.90
FSH (IU/L)	6.81	3.35
LH (IU/L)	3.77	2.10
E ₂ (pg/ml)	41.28	21.29
Inhibin B (pg/ml)	53.87	40.02
AMH (ng/ml)	2.23	1.90
Total AFC (n)	8.35	2.83
Mean ovarian volume	5.44	2.31

Table 2. Correlations of the number of antral follicles, the mean ovarian volume, AMH and the others ovarian reserve parameters

Parameters	AFC		The Mean Ovarian Volume		AMH	
	r	p	r	p	r	p
Age (years)	-0.40	<0.0001**	-0.18	0.03*	-0.18	0.03*
FSH (IU/L)	-0.29	<0.0001**	-0.27	0.001**	-0.29	<0.001**
LH (IU/L)	0.06	0.42	-0.05	0.94	0.13	0.11
E ₂ (pg/ml)	-0.08	0.31	0.07	0.34	-0.42	0.62
Inhibin B (pg/ml)	0.15	0.06	0.18	0.03*	0.25	0.002**
AMH (ng/ml)	0.46	<0.0001**	0.37	<0.0001**	1	

Discussion

The count of the number of antral follicles by ultrasonography is the best predictor for the quantitative aspect of ovarian reserve (22). There is no consensus on identification of the antral follicles (2), however several evidence based studies suggested to select the follicles as antral follicles based on a diameter measurement as 2 to 10 mm. (3-10). It has been reported that human antral follicles measuring <6 mm express the greatest amount of AMH, and that levels decline with antral follicles increase in size (23). Two to six mm antral follicles were defined as AFC in our study. We observed that serum AMH levels are strongly related to early AFC, with a significance that was remarkably stronger than age, serum levels of inhibin B, E_2 , FSH and LH. Similar results were found by the previous published studies about the relationship between AMH and antral follicle count and the coefficients of correlation were reported as stronger (0.71-0.74) than present study (0.46) (24, 25). The correlation of AMH and the different sizes of antral follicle was studied previously; the best correlation was found between AMH and >5 to 6 mm size of AFC and correlation coefficient (r) was reported as low as 0.41. These different results may be explained by the lack of an international assay standard for AMH measurements.

In our results; a negative relationship was observed between FSH and total AFC and the ovarian volume. These data confirms the hypothesis of a stimulating role of FSH on granulosa cells on the antral follicle, caused by the dependency of FSH levels on the negative feedback from E_2 and possible different regulation of AMH as compared with other hormonal parameters. Although little is known about FSH effects on AMH expression during the early follicular phase, it can be presumed that this hormone is less FSH-sensitive than inhibin B and E_2 .

On the contrary of previous reports; no correlations were detected between total AFC and inhibin B and E_2 levels in this study. This result could be explained by the modulator role of FSH for inhibin B and E_2 . During the luteal-follicular transition, the secretion of inhibin B and E_2 by the early antral follicles modulates their own stimulation by FSH (26, 27). This implies that inhibin B and E_2 levels depend not only on the bulk of active granulosa cells available, as represented by follicular number and sizes, but also on their stimulation by FSH. There are potential advantages of using AMH over AFC or the other parameters because AMH can be measured throughout the cycle, in contrast to the other parameters, which can only be determined in the early follicular phase (28, 29). Therefore, AMH may represent a more independent and reliable marker of early antral follicle activity than inhibin B and E_2 , and FSH on cycle day 3.

As a conclusion, our results indicate that serum AMH levels are strongly related with ovarian follicular status during the early follicular phase, and also this relationship is more significant than other ovarian reserve parameters. These results also indicate that, serum AMH measurement is better predictor for the number of early antral follicles than conventional hormone measurements. This point may be helpful to refine future clinical applications of AMH measurements in routine infertility work-up for evaluating the fertility potential and monitoring infertility treatments.

Conflict of interest

No conflict of interest is declared by authors.

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