

## SERUM LEVELS OF ANTIMÜLLERIAN HORMONE IN WOMEN WITH REGULAR MENSTRUAL CYCLES

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**SUMMARY** – Antimüllerian hormone (AMH) is produced by Sertolli cells of the testes and granulosa cells of the ovaries. Recent studies have indicated that AMH may be a novel measure of ovarian reserve. Also, earlier reports have presented minimal fluctuations of AMH levels throughout the menstrual cycle. The aim of this preliminary study was to demonstrate the relation of serum AMH levels and age in women with regular menstrual cycles and normal hormonal regulation of ovarian function. The study included 35 women divided into two groups of women aged 30 or younger and those older than 30. Hormone concentrations were assessed by measurements of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), testosterone (T), sex hormone binding globulin (SHBG) and AMH on cycle day 3-5 (follicular phase); and LH, FSH and E2 on cycle day 13-15 (ovulation). Progesterone level was determined on cycle day 19-23 (luteal phase). Median age differed significantly between the two groups of study subjects ( $P=0.001$ ). Study results confirmed regular ovarian response to physiological gonadotropin stimulation, which is the assumption for normo-ovulatory cycles. Some decrease in the mean serum AMH levels was recorded in women over 30 years of age, although the difference was not statistically significant ( $P=0.0693$ ). There was no statistically significant difference in serum AMH concentrations between follicular phase and ovulation in study women ( $P=0.3124$ ). Our preliminary results, although obtained in a limited number of women, support the diagnostic value of AMH as a reliable *marker of ovarian reserve*.

**Key words:** *Anti-Müllerian hormone; Fertility – physiology; Ovary – physiology; Menstrual cycle – blood; Biological markers – blood*

### Introduction

Antimüllerian hormone (AMH) is a glycoprotein, a member of the transforming growth factor- $\beta$  family. In men, AMH is produced by Sertolli cells of testes, from antenatal testicular differentiation up to puberty and adult age<sup>1</sup>. On the other hand, it is secreted by granulosa cells of women's ovaries, from puberty to

menopause<sup>1</sup>. In prepubertal girls, serum AMH levels are undetectable or very low, with maximal values detected after the onset of puberty<sup>2</sup>. The highest expression of AMH has been demonstrated in the stage of preantral and small antral follicles<sup>3</sup>. Positive correlation has been established between serum AMH levels and antral follicle count (AFC), and inverse correlation with increasing age. In menopausal women, AMH concentrations significantly decline, until they become almost undetectable in postmenopausal stage<sup>4</sup>. On the other hand, subtle changes in serum levels of follicle-stimulating hormone (FSH) occur with diminishing of the ovarian reserve, while sig-

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nificant elevation is observed when menstrual cycles have already become irregular. Consequently, AMH is a very sensitive marker of ovarian reserve because it reflects the number of follicles, activated from the existing pool of primordial follicles<sup>5</sup>. Furthermore, the advantage of AMH over other ovarian parameters is its independence of gonadotropins and stable serum concentrations during the menstrual cycle<sup>6-8</sup>.

On the trail of these findings, we have researched AMH levels in healthy females of reproductive age in our own population. The aim of this preliminary study was to demonstrate the relation of serum AMH levels with age in women with regular menstrual cycles and normal hormonal regulation of ovarian function.

## Subjects and Methods

Out of 52 female volunteers, 35 women were selected for the study, based on the inclusion criteria of age 18-45 years, regular menstrual cycles (30±2 days) within one year, and no hormonal therapy for at least three months. In addition, study subjects had no endocrine disorders that could interfere with ovarian function in their personal medical history. Women were recruited from February 2009 until April 2009 and they all provided a written consent for inclusion in the study. Data collection and analyses were performed at Laboratory of Endocrinology, University Department of Oncology and Nuclear Medicine, Sestre milosrdnice University Hospital, Zagreb.

Blood was collected by venepuncture during follicular, ovulation and luteal phases of the current menstrual cycle. Samples were centrifuged and sera were stored at -20 °C until analysis. Concentrations of

lutropin (LH), follitropin (FSH), estradiol (E2), testosterone (T), sex hormone binding globulin (SHBG) and AMH were measured on cycle day 3-5 (follicular phase), and LH, FSH and E2 on cycle day 13-15 (ovulation). Progesterone level was determined on cycle day 19-23 (luteal phase). We considered the cycle as ovulatory if the mid-cycle LH peak occurred as well as if the mid-luteal serum progesterone exceeded the concentration of 16 nmol/L<sup>10</sup>. All hormones except for AMH were assayed by electrochemiluminescence immunoassay (ECLIA, Roche Diagnostics GmbH) on Elecsys 210 analyzer. AMH concentrations were measured by enzyme-linked immunosorbent assay (ELISA, Diagnostic System Laboratories, Inc.).

Statistical analyses were performed using MedCalc<sup>®</sup> statistical software (MedCalc 9.3.9.0, Frank Schoonjans, Mariakerke, Belgium). The normality of distribution of parameters was ascertained by Kolmogorov-Smirnov test. Because of the small number of subjects, nonparametric analyses were applied; comparisons between groups were performed by Mann-Whitney test and Wilcoxon test for paired samples. The value of  $P < 0.05$  was considered statistically significant.

## Results

Out of the initial number of 52 women, 17 were excluded for the reasons inconsistent with the selection criteria. In 12 women, we found subnormal mid-cycle estradiol concentrations and/or absence of progesterone peak in luteal phase. There was one case of started pregnancy and four cases with uncompleted blood collection in the current menstrual cycle. Other 35 women

Table 1. Concentrations of hormones in follicular, ovulatory and luteal phases of menstrual cycles in 35 women of reproductive age

	LH (IU/L)	FSH (IU/L)	E2 (pmol/L)	T (nmol/L)	SHBG (nmol/L)	Free T (pmol/L)	P (nmol/L)
Follicular phase	5.6±3.0 (4.6-6.7)	7.1±2.0 (6.4-7.8)	174±64.7 (152-97)	1.0±0.5 (0.9-1.2)	73.9±28.8 (64-83.8)	11.4±5.5 (9.5-13.3)	-
Ovulatory phase	11.9±7.0 (9.5-14.3)	6.0±2.1 (5.3-6.7)	734±477 (570-898)	-	-	-	-
Luteal phase	-	-	-	-	-	-	48.4±19.5 (41.3-55.3)

Concentrations are expressed as mean ± SD, with 95% confidence interval (CI); LH = lutropin; FSH = follitropin; E2 = estradiol; T = testosterone; SHBG = sex hormone binding globulin; P = progesterone

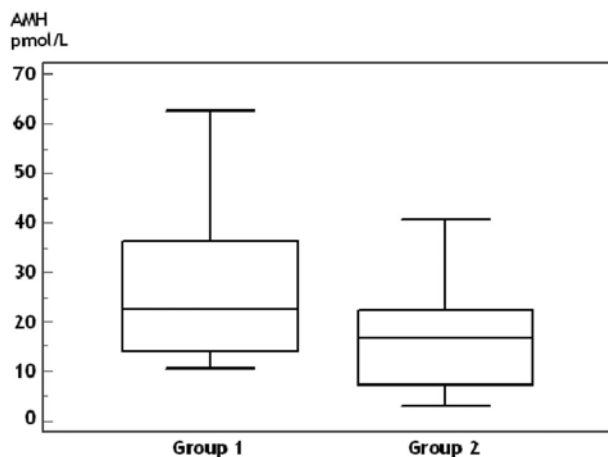


Fig. 1. Serum antimüllerian hormone (AMH) levels in women according to age (group 1:  $\leq 30$  years and group 2:  $> 30$  years).

had normal hormonal status and fulfilled inclusion criteria. Median age was 32 (range 19–44) years.

Concentrations of measured hormones in study women in different phases of the cycles are shown in Table 1. Concentrations of free testosterone were calculated on the basis of total testosterone, albumin and SHBG, according to the previously established formula<sup>10</sup>. Using Kolgomorov-Smirnov test, the results for all analytes fitted Gaussian curve, thus confirming compliance with normal distribution.

To estimate the relationship of AMH and age, women were divided into two groups of 15 women

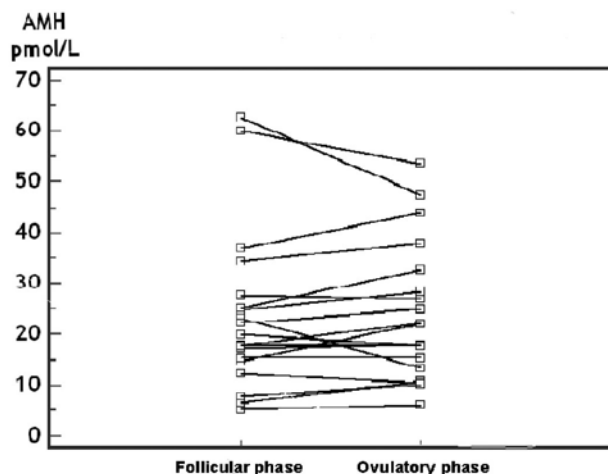


Fig. 2. Comparison of serum antimüllerian hormone (AMH) concentrations between the follicular and ovulatory phase of menstrual cycle ( $n=19$ ).

aged  $\leq 30$  and 20 women aged  $> 30$ . Median age differed significantly between the two groups (26 vs. 36;  $P < 0.0001$ ). Median value of AMH was 22.6 pmol/L (95% CI: 13.2–39.0) and 16.8 pmol/L (95% CI: 7.1–22.4), respectively, although the decrease was not statistically significant ( $P=0.0693$ ). Results of comparison between the two groups are shown in Figure 1.

We also compared E2 and FSH levels in the two groups of women during follicular phase using Mann-Whitney test. The mean E2 concentration was 162.5 and 178.5 pmol/L in the  $\leq 30$  and  $> 30$  age group, respectively. Serum FSH levels in the two groups were 6.4 and 7.5 IU/L, respectively. There was no statistically significant difference in the mean concentrations of E2 ( $P=0.423$ ) and FSH ( $P=0.243$ ), when the results in younger women were compared with those in older ones.

In addition, we compared serum AMH concentrations in 19 women during follicular and ovulatory phase. Median value in follicular phase was 20.1 pmol/L (95% CI: 14.8–27.5) and 22.2 pmol/L (95% CI: 13.7–32.4) in ovulatory phase, respectively. Using Wilcoxon test for paired samples, we found no significant difference in AMH concentrations between the two phases of the cycle ( $P=0.3124$ ) (Fig. 2).

## Discussion

Recent studies have indicated that AMH may be a novel measure of ovarian reserve<sup>1,2,4</sup>. In addition, when comparing AMH with other ovarian tests, it seems to be the best marker reflecting the oocyte/follicle pool. Substantially elevated serum levels of FSH are seen when cycles have already become irregular<sup>11</sup>. Due to the lack of a similar study in Croatia, our aim was to evaluate AMH as a parameter dependent of ovarian aging in a group of women in reproductive age of our own population. We are aware that regular menstrual cycle and normal serum levels of gonadotropins and ovarian steroid hormones are not sufficient to confirm the woman's reproductive health. Therefore, we had no intention to correlate AMH with fertility potential of study women, but to find the relationship of AMH with other parameters relevant for the assessment of ovarian function. The role of AMH in predicting ovarian response in women with decreased fertility, especially before treatment in the assisted reproduc-

tive technology (ART) procedures, has been studied profoundly<sup>1,5</sup>. Assuming that serum AMH concentration is a sign of follicular reserve exhaustion, we divided study women into two groups, arbitrarily taking the age of 30 as a boundary. Median age differed significantly between the two groups. We showed a certain decrease in the mean serum AMH levels in women over 30 years of age, although the difference was not statistically significant. In addition, we compared their FSH and E2 levels as serum parameters that are physiologically associated with ovarian aging. In both groups of women, we confirmed regular ovarian response to gonadotropin stimulation, which is the assumption for normoovulatory cycles. Also, median serum AMH concentrations in these women are in a range that reflects the preserved ovarian follicular pool<sup>12</sup>. As shown in a study performed by van Rooij *et al.*, AMH levels changed longitudinally over time in the same women divided into five-year classes<sup>2</sup>. Of course, we must take into account that the number of 35 women in our study was too small to make more narrow age groups and apply appropriate statistical analysis to get better insight in the subtle changes of all study hormones with aging.

Earlier reports describe minimal fluctuations of AMH levels throughout the menstrual cycle<sup>6-8</sup>. We found no statistical difference in serum AMH concentrations between follicular phase and ovulation in study women. Contrary to other ovarian reserve markers, the possibility of measurement of AMH at any time during the cycle is a great advantage in clinical practice<sup>13</sup>.

In conclusion, our results, although preliminary and based on a limited number of women, support the diagnostic value of AMH as a reliable marker of ovarian reserve. According to the latest report of the Croatian Institute of Public Health, more than 40% of Croatian women get pregnant and deliver babies after the age of thirty<sup>14</sup>. Even more, for several years we have also been faced with the fact that there is a constant decrease in the number of women that reach their first pregnancy in optimal period of reproductive life. Introducing the measurement of serum AMH in the assessment of women's fertility potential becomes one of the necessities for the novel and efficient diagnostic algorithm in our country as well.

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## Sažetak

## SERUMSKE RAZINE ANTI-MÜLLEROVA HORMONA U ŽENA S REDOVITIM MENSTRUACIJSKIM CIKLUSOM

I. Zec, D. Tišlarić-Medenjak, Ž. Bukovec-Megla, V. Harni i Z. Kusić

Anti-Müllerov hormon (AMH) sintetizira se u Sertolijevim stanicama testisa u muškaraca i granuloza stanicama jajnika u žena. Dosadašnje studije dokazale su važnost određivanja anti-Müllerovog hormona kao novog biljega u procjeni ovarijske rezerve. Također, ranije objavljeni rezultati pokazali su neznčajnu promjenjivost koncentracija AMH u serumu tijekom menstruacijskog ciklusa. Cilj ovoga preliminarnog istraživanja bio je ustanoviti odnos koncentracije AMH u žena s redovitim ciklusima i normalnim hormonskim statusom u odnosu na njihovu dob. Uključeno je 35 žena podijeljenih u skupine od 30 godina i mlađih te starijih od 30 godina. Hormonsku regulaciju menstruacijskog ciklusa smo potvrdili određivanjem koncentracija lutropina (LH), folitropina (FSH), estradiola (E2), ukupnog i slobodnog testosterona (T), proteinskog nosača spolnih hormona (SHBG) te AMH u folikularnoj fazi ciklusa (3.-5. dan). U ovulacijskoj fazi (13.-15. dan) određivali smo koncentracije LH, FSH, E2 i AMH, odnosno progesterona (P) u luteinskoj fazi (20.-23. dan). Medijani dobi u mlađoj i starijoj skupini bili su statistički značajno različiti ( $P=0,001$ ). Rezultati su potvrdili urednu hormonsku regulaciju djelovanja osovine hipofiza-jajnici u izabranih ispitanica. Ustanovili smo relativno niže koncentracije AMH u žena iznad 30 godina u usporedbi sa skupinom mlađih, iako razlika nije bila statistički značajna ( $P=0,0693$ ). Također, prosječne koncentracije AMH u folikularnoj fazi i ovulaciji nisu bile statistički značajno različite ( $P=0,3124$ ). Preliminarni rezultati ovoga istraživanja, iako na ograničenom broju ispitanica, podupiru dijagnostičku važnost AMH kao osjetljivog biljega u procjeni ovarijske rezerve.

Ključne riječi: *Anti-Müllerov hormon; Fertilitet – fiziologija; Jajnik – fiziologija; Menstruacijski ciklus – krv; Biološki biljezi – krv*

