Serum antimüllerian hormone in healthy premenopausal women

Antimüllerian hormone (AMH) is extensively studied in ovarian aging and pathology; however, little is known about correlates in healthy premenopausal women. We found that AMH levels are strongly inversely associated with age and differed significantly between oral contraceptive pill users and nonusers, whereas no significant associations were seen between AMH and other clinical, behavioral, and anthropometric characteristics and laboratory variables, making it an attractive hormone for clinical applications. (Fertil Steril® 2011;95:2718–21. ©2011 by American Society for Reproductive Medicine.)

Key Words: Müllerian-inhibiting substance (MIS), antimüllerian hormone (AMH), ovary, premenopausal, healthy

Antimüllerian hormone (AMH) is growth factor within the transforming growth factor-β family. In women, AMH is expressed by ovarian granulosa cells and controls the recruitment and growth of primary follicles. Levels correlate with the number of antral follicles, which predictably decline with age and disappear at menopause (1, 2). Levels of AMH do not fluctuate significantly across the menstrual cycle, and levels are independent of FSH, LH, and E_2 levels (1, 3, 4). These characteristics make AMH a unique ovarian hormone and an attractive target for study.

The role of AMH in various pathologic conditions, including polycystic ovary syndrome (PCOS) and ovarian granulosa cell tumors, as well as in IVF, has been well defined. Although AMH is extensively studied in these circumstances, little is known about its correlates in healthy women. The purpose of our study was to define associations in healthy premenopausal women between AMH concentration and anthropometric, behavioral, temporal, and seasonal characteristics, as well as serum concentrations of steroid sex hormones.

Subjects were selected from the control population in a prospective case–control study on the association of AMH and breast cancer risk (5) nested in the Columbia, Missouri Serum Bank. Participants were volunteers in the National Cancer Institute's Biological Markers Project who were identified primarily through the Breast Cancer Detection Demonstration Project at the University of Missouri Hospital and Ellis Fischel Cancer Center in Columbia, Missouri. All gave informed consent and donated blood to the bank between 1977 and 1987. Initial follow-up continued until 1989. The National Cancer Institute conducted an extended follow-up in 1999–2004. Women were eligible to be controls in the breast cancer case–control study if they were premenopausal at blood collection and had never been diagnosed with cancer other than nonmelanoma skin cancer (n = 258). Because the frequency of immeasurable AMH values increases dramatically as women approach menopause, women close to menopause provide limited information about clinical correlates of AMH levels. Thus, we limited our evaluation to the 135 women who were premenopausal and younger than 45 years. For 80% of women, data were available on start of last menses, and all of these women were within 33 days of the start of their last menstrual cycle at the time of blood collection. Because of our age restriction, we presumed that the remaining 20% without data on last menses also were premenopausal.

Demographic, anthropometric, and behavioral data were collected, including age, height, weight, menstrual and reproductive histories, smoking, hormone use, and family history of breast

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cancer. Approximately 30 mL of blood were collected from each woman and processed and stored using standard procedures.

At the initiation of our investigation, AMH was quantified by ELISA using a commercially available kit (Beckman-Coulter Diagnostics Systems Laboratories). The AMH interassay coefficient of variation averaged 8.2%, and its limit of detection (LOD) was 0.43 pmol/L.

The association of AMH with individual characteristics was evaluated using nonparametric statistics because of the extreme skewness of the data. Univariate tests of significance were performed using Wilcoxon rank-sum and Kruskal-Wallis tests. Analyses were age adjusted by including age as a continuous term in regression models. Adjusted medians were estimated using median regression with bootstrap variances. Tests for trend were performed by including quantile rank in models as an ordinal variable. Because AMH levels differed by oral contraceptive pill (OCP) use, analyses were repeated to control for OCP use and age. For evaluation of sex hormone levels, women taking OCPs and women with unknown menstrual cycle day were eliminated from the analysis. Additionally, a restricted cubic spline for cycle day, using the method of Harrell (6), was included in E_2 models to account for the variation of this hormone over the menstrual cycle. For women with AMH levels below the assay LOD, the value was imputed using 0.21 pmol/L, the midpoint between 0 and the assay LOD. Two-sided P values $<.05$ were considered statistically significant. All analyses were performed using Stata 10 (StataCorp).

All women were Caucasian, and their mean (±SD) age was 41 ± 2.48 years. Their mean body mass index (BMI) was 25 ± 5.15 kg/m^2. Sixty percent were normal weight, 24% were overweight, and 16% were obese. Ninety percent of the women were not using OCPs, and 96% were parous at the time of blood collection, with an average of 2.8 ± 1.36 children per woman. Blood collection occurred from 8:00 AM to 4:00 PM, and draws were evenly distributed throughout this time frame.

The median overall AMH level was 3.57 pmol/L (95% confidence interval [CI], 2.14–4.57 pmol/L). Antimüllerian hormone was strongly inversely associated with age (trend $P<.001$), declining from a median of 7.85 pmol/L in the youngest women (95% CI, 5.21–10.00 pmol/L) to 0.50 pmol/L (95% CI, 0.21–1.43 pmol/L) in the oldest (see Table 1). This substantiates previous findings that AMH concentration declines significantly with increasing age in premenopausal women (7–9). Over the age range of premenopausal women included in these analyses, a near-linear relationship between serum AMH level and age was observed.

After adjusting for age, AMH levels differed significantly between users and nonusers of OCPs ($P=.001$). The median AMH level in users was 3.00 pmol/L (95% CI, 2.07–3.86 pmol/L), compared with 4.57 pmol/L (95% CI, 3.86–5.21 pmol/L) in nonusers. These findings are consistent with a clinical trial in which OCPs lowered AMH levels (10) and one in which AMH concentration was found to increase during the hormone-free interval in OCP users (11). However, in two other small clinical trials, treatment with OCPs did not affect serum AMH levels in healthy women (12, 13) or in patients with PCOS (12). These discrepancies may also be related to the differences in OCP preparations, because those used in the late 1970s when our data were collected were stronger than those used recently (14). Notably, few patients were taking OCPs, therefore this association should be explored in a larger group of women. Additionally, adjustment for OCP use in addition to age did not alter any associations between AMH and other variables.

No significant associations were seen between AMH and anthropometric, menstrual, or reproductive characteristics, including BMI, smoking status, menstrual cycle phase, and temporal or seasonal characteristics of blood collection. Regarding BMI and AMH, conflicting data exist. Three previous studies show that healthy obese women have lower AMH levels compared with their nonobese counterparts (15–17); however, BMI was not associated with serum AMH concentrations in normo-ovulatory women undergoing infertility workups (18). The explanation of the discrepancy between the findings remains unclear. Regarding smoking, one report noted a difference in AMH concentration between smokers and nonsmokers, although this was only significant in late-reproductive and perimenopausal women, of whom 16% had undetectable AMH levels. The correlation was not present among premenopausal women not using OCPs (19). Similar to our findings, other reports agree that AMH concentration does not vary by menstrual phase (3, 20–23). This is in contrast to other ovarian hormones that display considerable cyclic variation. Our evaluation does not support the assertion that differences in findings across studies are due to timing of blood collection (24).

Associations between AMH and serum E_2, T, or sex hormone-binding globulin (SHBG) levels were not seen (data not shown). Estradiol, T, and their biological fractions generally increased with increasing AMH level, but tests for trend were not statistically significant. Regarding E_2, this is consistent with most studies (8, 22, 25–28). Antimüllerian hormone concentration was previously reported to be positively associated with T in healthy women (29), but results are not consistent across studies (28). In contrast, SHBG levels decreased with increasing AMH, but the trend was not statistically significant. In analyses that adjusted for menstrual cycle day or phase, AMH concentration was not significantly associated with sex hormones.

Investigators have reported on correlates of serum AMH levels in PCOS, IVF, and cancer patients. However, limited data exist for healthy women, and a major strength of our analysis is that it defines associations between AMH concentrations and characteristics of healthy premenopausal women. Antimüllerian hormone was analyzed in serum using an ELISA that has been shown to be valid and reproducible (3, 30). We also were able to take advantage of newer statistical methods for analyzing skewed data that are included in commercially available statistical software packages. There were also several limitations to our study. Our sample size may not have sufficient power to detect true associations that exist among certain variables. Point estimates for age at menarche, bioavailable E_2, bioavailable T, and SHBG suggest that there might be trends that would be uncovered if more samples were available for analysis. Although values of some variables of interest, such as BMI, were reasonably distributed across the expected range, for other variables distributions were limited. Few women were nulliparous, making conclusions regarding AMH and parity tenuous.

Antimüllerian hormone has many characteristics favorable for use in the clinical arena. Its independence of temporal,