Levels of antimüllerian hormone in serum during the normal menstrual cycle

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Objective: To determine whether levels of antimüllerian hormone (AMH) in serum vary during the normal menstrual cycle, using the most recently developed immunoassay method.
Design: Prospective cohort study.
Setting: Not applicable.
Patient(s): Women with normal menstrual cycles and between the ages of 18 and 45 years were recruited (n = 45). Blood samples were collected on 5 days within each cycle: two in the follicular phase and three after confirmed ovulation. Exclusion criteria were anovulatory cycles, incomplete sample collection, insufficient blood volume, or non-Caucasian ethnicity.
Intervention(s): None.
Main Outcome Measure(s): Serum samples were tested for levels of AMH using a new immunoassay method (Ansh Labs). The effects of body mass index (BMI) and smoking on serum AMH levels were considered.
Result(s): Serum AMH levels varied significantly during the menstrual cycle, with the highest levels in the follicular phase. When the analysis was stratified by age, AMH variation during the menstrual cycle was significant only for women older than 30 years. Serum AMH levels were not significantly altered by BMI or smoking.
Conclusion(s): The new AMH immunoassay revealed a follicular phase rise in serum levels, particularly in women over the age of 30 years. This is consistent with other reports finding an interaction of menstrual cycle variation in AMH and chronological age. Nonetheless, the extent of variation is small, and sampling on any day of the menstrual cycle is expected to adequately reflect ovarian reserve.
Clinical Trial Registration Number: ClinicalTrials.gov (www.clinicaltrials.gov) registration no. NCT01337999. (Fertil Steril® 2015;■:■–■. ©2015 by American Society for Reproductive Medicine.)
Key Words: Endocrinology, follicle development, luteal phase, ovarian reserve, müllerian inhibitory substance

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The pool of primordial follicles in the ovary declines as a natural function of aging in women (1). This decline in ovarian reserve is associated with a concomitant age-related decline in fertility (2). Assessment of ovarian age is of interest for women seeking to conceive by natural or assisted reproductive methods.

While chronological age is an indicator of ovarian reserve, other measures are more accurate. Among hormone markers tested, serum levels of antimüllerian hormone (AMH) have emerged to be most reflective of the ovarian status. AMH is produced by small primary, secondary, and preantral follicles (3), and serum levels are directly related to the primordial follicle pool (4).

A practical challenge regarding the use of AMH to assess ovarian reserve is the development of normative data. One issue that has been debated in the

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literature is whether or not there is variation in serum AMH levels relative to menstrual cycle day. About half of the published studies argue that AMH levels vary across the menstrual cycle, while the others suggest that there are no significant changes (reviewed in 5). Still others support the notion that the variability depends on the age range of the women being studied (6–8). Serum AMH levels can also be influenced by ethnicity (9, 10), body mass index (BMI) (11), smoking, and hormone contraceptive use (12).

To date, all studies of serum AMH across the normal menstrual cycle have employed the original immunoassay from Diagnostic Systems Laboratories, which is no longer available, or the Beckman Coulter Generation II method, modified recently to avoid interference of complement proteins. The objective of this prospective study is to determine AMH levels in women with regular menstrual cycles using a new and robust assay method (13, 14) and with consideration of demographic covariates.

MATERIALS AND METHODS

Subjects

Women with normal menstrual cycles, ages 18–45 year old, were recruited from the local community by the Center for Reproduction and Infertility, which is based in Providence, Rhode Island. Hormonal contraception was not used in the preceding 2 months for any subject. Information regarding past medical and surgical history, medications, and obstetrical and gynecologic history was collected. A physical examination including a pelvic exam was performed to exclude pelvic masses, and a negative urine pregnancy test was required.

Enrolled subjects had a total of five blood samples drawn in the menstrual cycle: two in the follicular phase on days 3 and 8–10 and three in the luteal phase, 1, 3, and 10 days after an LH surge. Monitoring for an LH surge was performed using the Clearblue Digital Ovulation Test (Swiss Precision Diagnostics). Ovulation was confirmed by serum P levels. Blood samples were centrifuged, and serum was separated and stored frozen (−80°C) for batch analysis. In a subset of subjects, urine was also collected on the day of each blood draw. Urine was decanted and stored frozen at −80°C.

Ethical Approval

All subjects gave informed consent for this study, which was approved by the Women and Infants Institutional Review Board for Human Studies.

Hormone Measurements

Hormone measurements in serum included LH, E_2, and P. Testing was performed using the automated ARCHITECT i2000SR instrument (Abbott Laboratories). Assay sensitivities were 0.5 mIU/mL for LH, 10 pg/mL for E_2, and 0.1 ng/mL for P. Levels of AMH were determined using the ultrasensitive method from Ansh Labs, with a sensitivity limit of 0.08 ng/mL. Interassay coefficients of variation were 2.9% at 0.82 ng/mL and 3.1% at 2.76 ng/mL. Each sample was run in duplicate and required to have an intra-assay coefficient of variation below 10%. Samples with nondetectable levels of AMH using the ultrasensitive method were retested with a more sensitive method (pico AMH, Ansh Labs), which has a lower limit of detection of 1.2 pg/mL (0.0086 pmol/L).

Statistical Analyses

Analyses were performed using SAS statistical software. Medians, ranges, and interquartile ranges were calculated as descriptive statistics. All statistical testing was performed on natural logarithm-transformed AMH to account for deviations from a normal distribution. Mean log values were compared by blood draw using linear regression for longitudinal data. Blood draw was modeled as categorical fixed effect. The within-patient covariance pattern was selected by the Akaike Information Criterion. Unstructured covariance was the best fit for log AMH. Changes over time by patient characteristics were tested by including product interaction terms in the models. Model diagnostics were examined to detect deviations from modeling assumptions. Between-draw comparisons were adjusted by Scheffe's method to account for multiple testing. Comparisons within each blood draw were performed by analysis of variance (ANOVA). The association between smoking and AMH was also examined after matching patients by age (±2 years). For each smoking patient, a nonsmoking patient was randomly selected. All *P* values presented are two-tailed.

RESULTS

A total of 71 women consented for the study. Hormone testing was performed on samples from 59 of these women, three of whom were later excluded because of an anovulatory cycle as determined by a luteal phase $P<3$ ng/mL. Three additional women were excluded because they did not complete all blood draws. Since very few women reported a non-Caucasian race, these were also eliminated, leaving a final sample size of 45 completed menstrual cycles. A subset of women also contributed a urine sample ($n = 22$) at the time of each blood draw. Demographic variables for the study subjects are presented in Table 1.

Urinary AMH Levels

Two full menstrual cycles (10 samples) were evaluated using the ultrasensitive and pico AMH assay methods. AMH was not detected in any urine sample tested (<6 pg/mL, data not shown).

Serum AMH Levels across the Menstrual Cycle

Levels of AMH in serum were successfully determined across the menstrual cycle using the ultrasensitive assay, except for in one woman for whom two samples had very low levels (<0.08 ng/mL, 45 years, nonsmoker, BMI = 26). These samples were both successfully measured as 0.03 ng/mL using the pico AMH assay.

AMH levels in serum varied significantly across the menstrual cycle, with peak concentrations observed in the late follicular phase. Luteal phase levels of AMH were lower