Quantifying the intraindividual variation of antimüllerian hormone in the ovarian cycle

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Objective: To quantify intraindividual variability of antimüllerian hormone (AMH) as analytical and biological coefficients of variation and assess the effects of variation on clinical classification.
Design: Retrospective cohort study.
Setting: Not applicable.
Patient(s): Thirty-eight women referred by general practitioners.
Intervention(s): None.
Main Outcome Measure(s): Total intraindividual variability (CV_W), analytical (CV_A) and biological variability (CV_I) for each woman and for AMH ranges: low (<5 pmol/L), reduced (5–10), moderate (>10–30) and high (>30 pmol/L), with calculation of proportion of women crossing clinical cutoffs and expected variability around each cutoff.
Result(s): Cycling women (n = 38) contributed 238 blood samples (average 6 samples each). The average total intraindividual AMH variability was 20% (range: 2.1% to 73%). Biological variation was 19% (range: 0 to 71%) and at least twice the analytical variation of 6.9% (range: 4.5% to 16%). Reclassification rates were highest in women with low (33%) or reduced AMH (67%) levels. Expected variations around the 5, 10, and 30 pmol/L cutoffs were 3–7, 7–13, and 20–40 pmol/L, respectively. In a woman with mean AMH in the 10–30 pmol/L range, the span of results that could occur was 7–40 pmol/L.
Conclusion(s): Total variation in AMH was 20%, and the majority of this was biological. Changes in AMH resulted in reclassification in 29% of women and occurred most frequently in those with low and reduced AMH. In cycling women, the variability in AMH should be considered by clinicians, especially if a result is close to a clinical cutoff. (Fertil Steril® 2016;106:1230–7. ©2016 by American Society for Reproductive Medicine.)
Key Words: AMH, analytical variation, biological variation

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Antimüllerian hormone (AMH) has an important role in the assessment of ovarian reserve and is arguably the most accurate and robust ovarian biomarker (1) available today, better than age, antral follicle count, or follicle-stimulating hormone levels, which rise late (2–6). Various cutoffs have been suggested to define reduced ovarian reserve or increased risk of hyperstimulation during fertility treatments (7–10).

Although AMH is widely used in fertility assessment, there have been calls for caution with its use (5, 11) and appeals to consider the variability of results when assessing patients (12). Clark et al. (11) pointed out the need for care with use of absolute values and cutoffs and advised interpreting AMH in the context of other findings. Leader and Baker (12) also noted that AMH results can have dramatic variability due to common biological variations and that a careful approach to interpretation in the context of certain medications, assay changes, and calibration is required.

In clinical chemistry laboratories the importance of defining variation in a measurement is well recognized (13). The components of variation usually quantified include analytical and

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biological variation—the latter of which may in turn comprise both intra- (CV_I) and inter- (CV_G) individual variation (14). Biological variation encompasses the many exogenous and endogenous influences that can result in fluctuation of a result from day to day in a healthy individual (15).

Many factors are now known to influence AMH levels and may contribute to variation in an individual's results. Small changes in AMH (~10%) may occur due to circadian variation (16) and seasonal changes. An 18% reduction of AMH in winter (17) has been reported, with this reduction prevented by cholecalciferol supplementation. Reduction of AMH can occur with smoking (18, 19), acute illness (20), and diseases such as lupus (21). It has been suggested that considering general health when interpreting ovarian function and AMH may be important (1). In the study by van Dorp et al. (20), reduced AMH was correlated with other surrogate markers of general health such as temperature, C reactive protein, and hemoglobin.

As gonadotropins change continuously in cycling women, the concept that AMH may be at least partially gonadotropin responsive (22) may also explain some biological variation. Gonadotropins may have a role in stimulating the growth of follicles dependent on follicle-stimulating hormone and AMH levels (23), and it has been reported that AMH can be misleading in states of hypogonadotrophic hypogonadism (23, 24). Gonadotropin receptor hormone agonists can have a variety of effects, including suppression of AMH (22, 25) or suppression followed by a rising AMH (26). Oral contraceptives, which down-regulate gonadotropins, have been shown to reduce AMH levels (19, 27–31). Similarly, in pregnancy where gonadotropin suppression occurs, AMH is reduced (19, 32). Small changes in AMH throughout the menstrual cycle have also been documented (33–38), with a peak in AMH during the midfollicular phase and nadir periovulation (38). These cyclic changes are concordant with data showing that AMH gene expression and total AMH protein increase in follicles up to 8 mm and that these follicles contribute approximately 60% to serum AMH levels (39). Further, there is a sharp decline in AMH within a follicle larger than 8 mm as it is selected for dominance in the preovulatory phase (39).

Considering the variety of factors influencing AMH levels, the reminders to reflect on the variability of this hormone are very relevant (11, 12). Although individual influences on AMH appear minor, the overall effect on total variation of AMH has not been quantified. Our study assessed intraindividual variability of AMH (CV_W) in women presenting for community-based ovulation tracking. We determined intraindividual biological variability (CV_I) by collecting samples on different days of the cycle in the same woman, and analytical variability (CV_A) by analyzing the precision of AMH at various levels in patient pools. The impact of this variability on clinical cutoffs and categorization of women was also determined.

MATERIALS AND METHODS

Participants and Samples

This study was approved by the institutional review board of Joondalup Health Campus (Ethics Committee Approval No. 1218). Women (n = 41) referred by their general practitioner for pathologist-managed laboratory ovulation tracking by measurement of reproductive hormones gave consent to participate in the study. Tracking samples were collected in the morning from 7:30 AM to 9:30 AM as per routine laboratory practice to facilitate early transport and analysis for daily management of results. The women who were undergoing tracking sampling donated any remaining serum (after completion of their testing) to test AMH on different days of the menstrual cycle. The samples were collected over 1 to 3 consecutive months in all women, with nine women having at least two samples or more in two to three cycles of data. Women with evidence of ovarian cycling (determined by a rising estradiol to at least 500 pmol/L with concurrent luteinizing hormone surge (LH $\geq$20 U/L), and a minimum threefold increase in LH from baseline) (40–42) were included in the study. We excluded three women who did not have an LH surge and did not show evidence of biochemical cycling during the tracking sampling period. No women were on exogenous estrogens or progesterone therapy.

For statistical purposes we normalized the follicular phase in each woman to a 14-day length (43) and defined the phases of the cycle measured from midcycle (day of LH surge: day 0). The follicular phase was defined as days −14 to −1 and the luteal phase as days +1 to + 14 according to Hehenkamp et al. (44). A sample size of 38 women or more was calculated to provide sufficient power to estimate the total CV_W with a 95% confidence interval of total width 0.1 (or 10%) using the accuracy in parameter estimation approach.

Assays

Blood was collected into 5-mL serum separator tubes (BD Vacutainer; Becton Dickinson), allowed to clot at room temperature, and then centrifuged within 30 minutes at 1,200 × *g* for 10 minutes. Samples were analyzed within 1 to 3 hours for routine ovulation tracking (estradiol, progesterone, and gonadotropins), and aliquots were prepared for other routine tests. The remaining serum was decanted and frozen at −20°C until analysis of AMH in duplicate, according to the manufacturer's guidelines, using the AMH Gen II enzyme-linked immunosorbent assay (ELISA) from Beckman Coulter (Beckman Coulter).

Classification of Low, Reduced, Moderate, and High AMH Groups

Based on the previous literature (7–10) and current laboratory data, cutoffs were chosen at 5, 10, and 30 pmol/L to establish four AMH groupings: low (<5 pmol/L), reduced (5–10 pmol/L), moderate (>10–30 pmol/L), or high (>30 pmol/L) mean AMH, which corresponded to low ovarian reserve, reduced ovarian reserve, normal reserve, and risk for hyperstimulation, respectively. The results throughout the cycle for each woman were compared to these cutoffs (using the Beckman Gen II values).