The reproducibility of serum anti-Müllerian hormone in subfertile women: within and between patient variability

Serum anti-Müllerian hormone concentrations vary significantly over time and this should be taken into account when tailoring treatment protocols for patients undergoing controlled ovarian hyperstimulation (COH). Compared with FSH, serum anti-Müllerian hormone may have greater discriminatory power because of its modest intrapatient variation and the larger interpatient variation. (Fertil Steril® 2011;95:1185–7. ©2011 by American Society for Reproductive Medicine.)

Key Words: Anti-Müllerian hormone, FSH, sample variation, ovarian stimulation protocol

Available evidence suggests that basal anti-Müllerian hormone (AMH) is a reliable predictor of ovarian response to controlled ovarian hyperstimulation (COH) during IVF cycles (1–3). Hence measurement of circulating levels of AMH has been introduced into clinical practice and knowledge of the AMH level used to individualize the stimulation protocol (4). Nevertheless, the intercycle and intracycle reproducibility of AMH measurements within the same patient remains a matter of much debate. The published studies that have attempted to assess the variability of AMH suffer from methodological issues and present contradictory results (5–13). The aim of this study was to investigate the variation between repeated AMH measurements in the same patient and between patients and to compare AMH variability with that of serum FSH in the same patient group.

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A total of 186 women who had repeated AMH determinations as a part of routine investigations for subfertility at St. Mary's Hospital, Manchester, between September 2008 and November 2009 were selected. Variability in circulating AMH concentration was calculated and compared with variation in FSH. The study was approved by the Local Research Ethics Committee (UK-NHS 10/H1015/22), and written informed consent was obtained from all patients.

Blood samples were randomly taken during the menstrual cycle when patients attended the clinic for the routine work-up. The reasons for repeating AMH measurements were: [1] clinic's protocol for updating results every 6 months, [2] patient's request, and [3] clinician not aware of the initial AMH measurement. Serum for assay of AMH was separated within 2 hours from venepuncture and frozen in aliquots at -70°C until analyzed in batches.

The assays were handled and processed according to the manufacturers' recommendations. The AMH was measured by an enzymatically amplified two-site immunoassay (DSL Active MIS/AMH ELISA; Diagnostic Systems Laboratories, Webster, TX). The assay had a working range up to 100 pmol/L with minimum detection limit of 0.63 pmol/L. The intra-assay coefficient of variation (n = 16) was 3.9% (DSL low control) and 2.9% (DSL high control). The interassay coefficient of variation (CV) (n = 60) was 4.7% (DSL low control) and 4.9% (DSL high control). Each AMH sample was analyzed in duplicate, the mean of the two replicates being reported as the final result.

Basal FSH (days 3–5 of the cycle) was measured in the same study population (n = 186). Serum FSH concentrations were determined using the Cobas FSH assay (Roche Diagnostics, Germany), measured using an automated system (E170 autoanalyser; Roche Diagnostics, Mannheim, Germany). The intra-assay and interassay CV were 6.0% and 6.8%, respectively. Only a single replicate for FSH was analyzed.

The AMH levels are allocated to five bands with differing ovarian reserve, as previously suggested, (7): ≤2.2 pmol/L, very poor

ovarian reserve; >2.2 to ≤15.7 pmol/L, low ovarian reserve; >15.7 to ≤28.6 pmol/L, satisfactory (i.e., normal) ovarian reserve; >28.6 to ≤48.5 pmol/L, high ovarian reserve; >48.5 pmol/L, suggestive of polycystic ovary syndrome (PCOS).

Clinical data on 186 women were collected retrospectively using the electronic clinical database (AcuBase; NSR Software Solutions Ltd., United Kingdom). Previous gynecological history was obtained from written patient records. This included regularity of menstrual cycles, the day of the menstrual cycle when blood samples were collected, previous adnexal surgery, use of any hormonal treatment, and number of previous IVF cycles.

Data were analyzed using the *R* statistical environment (14) and the variability of AMH and FSH assays was computed on a logarithmic scale and expressed as a CV and transformed back to give an SD for the mean level. The sample, replicate, and patient variability were computed directly using a nested random effects model on the log-transformed values, and the significance of the effects tested using likelihood ratio tests. Agreement on categorization of various cutoff levels was estimated by Cohen's Kappa test (equally weighted). The limits of agreement in AMH and FSH variations were computed following Bland-Altman (15). These are based on log-transformed data and expressed as percentages—the limits on the measured scale will increase with increasing values. The effect of clinical parameters on the AMH levels were assessed after adjusting for age using a random effect model with age and the parameters as fixed effects and a patient random effect.

Women in the study were between 22 and 41 years old (mean age 33 years), had a body mass index (BMI) less than 30 kg/m^2, and were nonsmokers. The majority of the subjects and their partners had idiopathic subfertility (n = 76). Known subfertility factors included anovulation (n = 26), tubal factor (n = 36), and male factor infertility (n = 48).

A total of 386 AMH samples from 186 patients (mean number of samples 2.1 per patient) were collected. Thirteen samples were excluded because of hemolysis; data on the remaining 373 was analyzed. The median time between samples was 2.6 months, with a maximum of 12.7 months. The CV between replicates of the same AMH sample was 4.8%; between samples taken from the same patient at different times the CV was 28% and between samples taken from different patients the CV was 94% (Table 1). The variability terms between samples and patients were statistically significant ($P<.001$). Repeated AMH values in 42 of 172 women (24%) decreased into different (but adjacent) clinical categories when compared with the first AMH sample. The estimate of agreement (Cohen's Kappa) on the categorized AMH levels was 0.74 (95% confidence interval [CI] 0.59–0.90).

An age-related decline of 5.1% ± 1.5% ($P<.001$) per year in AMH levels was observed. The AMH measurements did not appear to be significantly affected by the stage of the menstrual cycle, presence of ovarian cysts, recent ovarian surgery, treatment with the combined oral contraceptive (OC) pill, or after IVF treatment.

A total of 325 early follicular phase samples from 186 subjects were analyzed for FSH (mean number of samples 1.7 per patient). The median time between samples was 4.7 months, with a maximum of 26 months. The CV between basal FSH levels from the same patient and between all patients was 27% and 30%, respectively (Table 1). The limits of agreement for repeat sampling, expressed in percentage terms, for AMH and FSH were ±55% and ±53%, respectively.

Although our results reveal the existence of variation in AMH measurements, we found no clinical factors that accounted for this variability and the data available are not sufficiently comprehensive to investigate the possible causes of this variation. Because the observed intra-assay variation is only 5%, we believe that the most probable explanation for the much larger overall variability (28%) is true biological variation in levels of circulating AMH in women. The observed variation in measured AMH concentrations may also have resulted from differences in sampling or in sample handling especially during the critical period between collection of the sample and separation of serum before analysis.

In spite of the limited evidence available on reproducibility of AMH, it is generally believed that AMH levels do not change between menstrual cycles (intercycle) or during the cycle (intracycle). The intercycle variability of AMH in women (n = 47) having spontaneous menstrual cycles has been investigated by Fanchin and colleagues (13). The investigators demonstrated that, during 3 consecutive months, basal AMH levels (intraclass correlation coefficient 0.89, 0.83–0.94) were more reproducible than both basal FSH (0.55, 0.39–0.71; $P<.01$) and antral follicle count (0.73, 0.62–0.84; $P<.001$). Although the study provides important data on short term cycle-to-cycle variability of AMH levels taken during early follicular phase, the results cannot account for our observed variability. Van Disseldorp and colleagues (16) examined intercycle variability of AMH measurements in women undergoing clomiphene citrate (CC) challenge tests or exogenous FSH ovarian reserve tests. The study showed that AMH was reproducible during four consecutive cycles. Other investigators (17) showed that AMH levels may be affected by administration of

TABLE 1

Variability of AMH and FSH measurements between patients, between samples, and between replicates.

	AMH			FSH		
Comparison	**Mean**	**SD**	**CV**	**Mean**	**SD**	**CV**
Between patients	12.7	12.0	94%	7.4	2.2	30%
Between samples	—	3.6	28%	—	2.0	27%
Between replicates	—	0.61	4.8%	—	Nd	Nd

Note: AMH measured in pmol/L, FSH in IU/L. AMH = anti-Müllerian hormone; CV = coefficient of variation; nd = not determined.

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exogenous gonadotropins but, as the majority of women have AMH measurements before fertility treatment, it is more relevant to establish whether there are fluctuations in AMH levels between spontaneous cycles.

Although intracycle AMH variability (within the same menstrual cycle) has previously been examined, it is difficult to draw convincing conclusions because of small study sizes, the contradictory results, and some methodological weaknesses (18). Four studies with a total of only 108 subjects concluded that there is no significant fluctuation in serum AMH levels in ovulating women throughout the menstrual cycle (5–8). Interestingly, the pooled results of four other studies with a total of 88 subjects demonstrated that there is considerable fluctuation in levels of AMH during the menstrual cycle (9–12).

Variation in circulating AMH concentrations compares well with other markers of ovarian reserve, despite the significance of its variation (13). In our study, although the intrapatient variability of AMH measurements was similar to that of FSH, the between patient variability of AMH was much greater compared with FSH. This suggests that AMH may have greater discriminatory power and allows clinicians to categorize patients into different groups of ovarian performance in COH. The retrospective design has to be considered as a limitation of our study.

In conclusion, we have observed a clinically and statistically significant variation in AMH measurements that is not attributable to any patient or cycle characteristics. It is plausible to suggest that this variation should be taken into account when developing triage algorithms for assigning patients to different COH protocols.

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