Variation in antimülllerian hormone concentration during the menstrual cycle may change the clinical classification of the ovarian response

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Objective: To assess the variability of antimülllerian hormone (AMH) concentrations in women with “adequate ovarian reserve” during unstimulated menstrual cycles and to determine the impact on clinical classifications.

Design: Pilot cohort study.

Setting: Private fertility clinic.

Patient(s): Twelve consecutive women (aged 29 to 43 years) referred to a fertility service, with AMH measurements repeated throughout unstimulated cycle, and who had at least one AMH measurement indicating “adequate ovarian reserve.”

Intervention(s): None.

Main Outcome Measure(s): AMH concentrations assessed in 82 serum samples from 12 women compared against the published cutoffs for reduced ovarian reserve and for risk of excessive response to ovarian stimulation.

Result(s): Over half the women (7 of 12) were reclassified as a result of testing AMH concentrations at different phases of the menstrual cycle. Over one-third (4 or 5 of 12) crossed a cutoff for reduced ovarian reserve; 2 of 12 crossed cutoffs predicting hyperstimulation. There was a statistically significant change in AMH concentration according to the day of the cycle, with a negative trend of the median AMH concentration from the follicular to luteal phase. The maximum change in median AMH concentration over cycle was 7.9 pmol/L, and the mean difference between the maximum and minimum AMH was 6.7 pmol/L.

Conclusion(s): In this pilot study, the AMH concentration varied during menstrual cycle, and the clinical classification of the ovarian response was altered. (Fertil Steril 2013;99:1791–7. ©2013 by American Society for Reproductive Medicine.)

Key Words: AMH, menstrual cycle

Discussion: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/hadlown-amh-menstrual-cycle-ovarian-response/

Over recent years antimülllerian hormone (AMH) has been shown to be a useful marker of ovarian reserve, and its level is now widely used in fertility therapy for individualizing stimulation protocols (1–4). Antimülllerian hormone has been shown to be better at reflecting declining ovarian reserve than age, antral follicle count (AFC), or the levels of follicle-stimulating hormone (FSH) or inhibin B (2, 5–7). Basal AMH has been shown to predict both excessive and poor response to stimulation with exogenous gonadotropin in women undergoing in vitro fertilization (IVF) (3, 4). The use of AMH to individualize treatment strategies for controlled ovarian stimulation has been shown to result in reduced clinical risk, optimized treatment burden, and maintained pregnancy rates (4). Also, AMH has been shown to be useful in identifying a good prognosis group for IVF and gonadotropin stimulation in women of advanced reproductive age (8).

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IVF have been defined (3, 4, 8, 9) and can help guide clinical management.

Despite the increasing importance of AMH in these clinical settings, there have been conflicting data about whether AMH varies in different states of healthy physiology, such as at different stages of the menstrual cycle (1). Earlier reports suggested that AMH was stable throughout the menstrual cycle (5, 10–12), but more recent studies have not always supported these claims. Sowers et al. (13) reported that the “aging ovary” did not show much variation in AMH throughout cycle, but in younger women there was significant variation in AMH during the menstrual cycle. Cook et al. (14) and Wunder et al. (15) also found that in young women significant changes occurred in AMH levels throughout the cycle, with higher levels noted during the follicular phase as compared with luteal phases. Another study noted changes in AMH through the cycle that were statistically significant, but the investigators argued that these were not sufficiently large to be clinically relevant (16). All these studies were conducted in women with normal menstrual cycles (13–16).

A large data set of AMH results (n = 3,260) published by Kelsey et al. (17) validated the levels of AMH in healthy women and identified inflection points in ovarian follicle dynamics. The peak AMH levels occurred at 24.5 years of age, with declining levels thereafter. The decrease in AMH with advancing age to undetectable levels occurs 5 years before the final menstrual period (2) and appears to occur before changes in other age-related variables such as FSH, which rises late (16, 18). However, the large data set of Kelsey et al. (17) suggested that two-thirds of the variation in AMH in healthy women was related to factors other than age, and others have noted that AMH varies markedly in infertile populations (2). The most widely accepted role of AMH has been in individualizing fertility treatment, but the variability of AMH in this population throughout cycle is yet to be well studied.

Our pilot study determined whether AMH changed significantly throughout the menstrual cycle in women being tested as part of a standard initial assessment at a fertility clinic. We also investigated whether any variation detected in AMH levels affected the clinical classification of a woman’s likely ovarian response. The levels of AMH were remeasured in women undergoing tracking of spontaneous, unstimulated cycles via routine blood collection in the follicular, ovulatory, and luteal phases of their cycle to determine the variability of the results. In women with at least one AMH measurement indicating “adequate ovarian reserve” (4), the results during the cycle were assessed against four published criteria (3, 4, 8, 9) to predict reduced or excessive ovarian response to IVF. We determined the proportion of women crossing the clinical cutoffs during the cycle.

MATERIALS AND METHODS
Participants and Samples

Fourteen consecutive women attending a fertility clinic who were having a natural, unstimulated cycle monitored were included in this study. Ethics approval to conduct this study was given by the Joondalup Health Campus Human Research Ethics Committee. Blood was collected during the follicular and periovulatory phases (selected days to enable identification of the day of ovulation) and luteal phase (7 and 14 days after ovulation) for each cycle. Between five and nine blood samples were collected for each woman over the cycle, and they were analyzed daily for luteinizing hormone (LH), FSH, estradiol, and progesterone. Serum samples were frozen within 4 hours of collection for AMH analysis. Two women with repeated AMH levels that indicated reduced ovarian reserve (<6.4 pmol/L) (4) were excluded, leaving 12 women for further analysis. There were 82 serum samples analyzed for AMH from 12 women (between five to nine samples per woman) with adequate ovarian reserve, across different phases of the menstrual cycle.

Day and Phase of Cycle

The follicular phase was normalized for statistical purposes to a 14-day length (19) according to the following formula:

\[ \text{Adjusted day} = \frac{\text{Actual day} \times (14/\text{Actual day of ovulation})}{1} \]

The adjusted day of the follicular phase was recorded for each woman. Ovulation was nominated as day 0, with the phases described according to Hehenkamp et al. (20) as early follicular (up to −10 days), mid-follicular (days −9 to −6), late follicular (days −5 to −2), periovulatory (days −1 to +1), midluteal (days +5 to +8), and late-luteal (days +9 to +14).

Assays

Blood was collected into 5 mL serum separator tubes (BD Vacutainer; Becton Dickinson), allowed to clot completely at room temperature, and then centrifuged within 30 minutes at 1,200 × g for 4 minutes. Serum was analyzed directly on a daily basis for estradiol, progesterone, FSH, and LH using the Siemens Centaur CP automated analyzer (Siemens HealthCare Diagnostics Pty Ltd.), and daily quality control (QC) samples were run at three levels (Lyphochek Immunoassay Plus Control; Bio-Rad Laboratories). All assays had between-assay variations of <12.5% for the low-level QC, and <5.1% for the medium and high level QC.

For AMH measurements, serum was decanted and frozen at −20°C within 4 hours, and study samples were subsequently analyzed together to minimize interassay variation. The laboratory performs the AMH assays four to five times per week depending on workload. The AMH assays were performed in duplicate according to the manufacturer’s guidelines using the AMH Gen II enzyme-linked immunosorbent assay (ELISA) from Beckman Coulter (Beckman Coulter Ireland). For AMH, QC materials (QC I and II) were used as directed by the manufacturer, and “in-house” patient pools at 7.7 pmol/L (low AMH pool) and 28.9 pmol/L (high AMH pool) were run with every assay. The precision using QC and patient pools between runs over 6 months was 4.2% and 3.8%, respectively, for QC I and II, and 5.5% and 4.6% for the low-AMH and high-AMH patient pools. All patient samples were analyzed in duplicate, and the results were calculated as the mean of duplicates.
Definition of Reduced Ovarian Reserve and Possible Excessive Response

Results throughout the unstimulated cycle for each woman were compared with four published cutoffs for reduced ovarian reserve (3, 4, 8, 9) and two cutoffs for possible hyperstimulation (3, 4), with the cutoffs converted to Beckman Coulter Gen II assay results in pmol/L when required (21). The AMH values in ng/ml or μg/L were multiplied by 7.143 to convert to pmol/L (4), and then the DSL assay results were converted to Gen II results (21) using the following equation:

\[ \text{AMH Gen II} = 1.40 \times \text{DSL} - 0.62 \text{ pmol/L} \]

In Beckman Coulter assay values, the cutoffs suggested for a reduced ovarian reserve include <6.4 pmol/L (4), ≤8.1 pmol/L (9), ≤8.6 pmol/L (8), and <9.4 pmol/L (3). Cutoffs for increased risk of excessive response included >20.4 pmol/L (4) and >36.8 pmol/L (3). The proportion of women crossing these cut-points during a cycle was determined.

Statistical Analysis

A total of 82 samples were collected throughout the cycle, with each woman contributing a minimum of five samples (five to nine per woman). Power calculations indicated that a minimum of five individuals with data at each phase would provide at least 80% power to detect differences in the transformed AMH response of 0.03 or more, assuming a 5% level of statistical significance.

Spearman correlation coefficients were calculated between AMH and other analytes, and age and day of cycle. Linear mixed effects models were used to explore the relationship between AMH and day of cycle, fitting random intercepts for each woman (22). The AMH measurement was power transformed (AMH^{0.3}) to ensure the statistical validity of the models. As successive measures on the same woman were expected to be highly correlated, each model incorporated an autoregressive correlation structure to account for the individual autodependence of measurements. A lag 1 correlation (AR1) was found to be significant and optimal to characterize the autodependence present. Any AMH variation was explored against the day of cycle, first fitted as a simple linear fixed effect, and second to assess the presence of cyclic behavior in AMH, with trigonometric fixed effects in the model. The cyclic behavior of estradiol, progesterone, LH, and FSH was confirmed by applying the same steps. Each variate was log transformed before subsequent analysis. The optimal fit of models with periodic terms was ensured by adjustment for phase shift (φ), according to the rule \( \phi = \arctan(\beta_{\cos}/\beta_{\sin}) \), where \( \beta_{\cos} \) and \( \beta_{\sin} \) were the trigonometric coefficients from a preliminary model (23, 24). This ensured that each variate was modeled around its individual acrophase.

Linear mixed models were also used to compare [1] transformed AMH concentrations across phases of the menstrual cycle by replacing day of cycle with a six level factor corresponding to the phases described previously, and [2] between preovulation and postovulation measurements. Finally, the proportional change in AMH was assessed by comparison with an intraindividual mean baseline.

The intraindividual and interindividual contributions to the variation of AMH and other hormones were evaluated with variance components analysis. Adjustment for age was made in all regression models, and the 5% significance level was used. We adjusted for multiple comparisons in the phasic model by setting the significance level at \( \alpha' = 0.05/5 = 0.01 \). All P values reported are from linear mixed effects models, as described earlier, unless otherwise indicated. Analyses were completed in the R statistical computing environment (R version 2.15.1) (25).

RESULTS

Participants

Twelve consecutive women with at least one AMH measurement indicating adequate ovarian reserve (>6.4 pmol/L) (4) were included in this study. Their ages ranged from 29 to 43 years, with three women aged over 40 years; the clinical details are provided in Table 1. The women had at least five or more samples collected each, for a total of 82 samples available for analysis. All the women had spontaneous cycles and ovulated, and none fulfilled the Rotterdam criteria for polycystic ovary syndrome.

Estradiol, Progesterone, LH, and FSH

The estradiol, progesterone, LH, and FSH levels showed statistically significant cyclic behavior (all \( P < .001 \)), as shown in Figures 1A and B. The variance between women and within each woman was calculated for each analyte using regression models. The within-individual contribution to variance was estimated against the day of cycle, fitting random intercepts for each woman (22). The AMH measurement was power transformed (AMH^{0.3}) to ensure the statistical validity of the models. As successive measures on the same woman were expected to be highly correlated, each model incorporated an autoregressive correlation structure to account for the individual autodependence of measurements. A lag 1 correlation (AR1) was found to be significant and optimal to characterize the autodependence present. Any AMH variation was explored against the day of cycle, first fitted as a simple linear fixed effect, and second to assess the presence of cyclic behavior in AMH, with trigonometric fixed effects in the model. The cyclic behavior of estradiol, progesterone, LH, and FSH was confirmed by applying the same steps. Each variate was log transformed before subsequent analysis. The optimal fit of models with periodic terms was ensured by adjustment for phase shift (φ), according to the rule \( \phi = \arctan(\beta_{\cos}/\beta_{\sin}) \), where \( \beta_{\cos} \) and \( \beta_{\sin} \) were the trigonometric coefficients from a preliminary model (23, 24). This ensured that each variate was modeled around its individual acrophase.

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<table>
<thead>
<tr>
<th>Age (y)</th>
<th>Infertility</th>
<th>Clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>Primary</td>
<td>Endometriosis</td>
</tr>
<tr>
<td>32</td>
<td>Primary</td>
<td>Vaginismus</td>
</tr>
<tr>
<td>34</td>
<td>Primary</td>
<td>Unexplained</td>
</tr>
<tr>
<td>35</td>
<td>Secondary</td>
<td>Male factor infertility</td>
</tr>
<tr>
<td>36</td>
<td>Primary</td>
<td>Husband had chemotherapy hence using cryopreserved semen</td>
</tr>
<tr>
<td>36</td>
<td>Primary</td>
<td>Previous ovarian cysts but not polycystic ovary syndrome</td>
</tr>
<tr>
<td>36</td>
<td>Primary</td>
<td>Unexplained</td>
</tr>
<tr>
<td>37</td>
<td>Secondary</td>
<td>Had laparoscopic sterilization and endometrial ablation due to heavy periods after last child; had failed reversal</td>
</tr>
<tr>
<td>38</td>
<td>Secondary</td>
<td>Amenorrhea subsequent to prolonged use of depot contraceptive; withdrawal bleed induced with oral contraceptive, then normal cycles resumed; spontaneously pregnant 5 mo after present study</td>
</tr>
<tr>
<td>41</td>
<td>Secondary</td>
<td>Regular 31–35-d cycle; one live birth and three miscarriages before study</td>
</tr>
<tr>
<td>42</td>
<td>Secondary</td>
<td>Regular 35-d cycle with occasional dysmenorrhea; twin pregnancy by IVF 9 mo. after present study</td>
</tr>
<tr>
<td>43</td>
<td>Secondary</td>
<td>Last child 15 y previous; IUCD for 10 y, trying for 2 y</td>
</tr>
</tbody>
</table>

TABLE 1

Clinical characteristics of the 12 women included in the study.
very high (~100%) for estradiol, progesterone, and LH. The within-individual contribution to variance for FSH was far less (83%), indicating slightly less than 20% interindividual variation.

**AMH throughout the Cycle**

There was a statistically significant change in AMH level with day of cycle between days −14 to +14 (β = −0.008 for AMH^0.3, P < .001) as shown in Figure 1C, with a negative trend noted in AMH from day −14 to +14 (β −0.001). Levels of AMH were statistically significantly higher in the early follicular phase than late luteal phase (β = −0.20 for AMH^0.3, P = .003). Levels of AMH fell from a median concentration of 15.0 pmol/L (mean 14.0) in the early follicular phase to a median of 7.1 pmol/L (mean 9.2) in late luteal phase, an overall difference of 7.9 for median values and 4.8 pmol/L for mean levels. When all follicular and luteal results were compared, the AMH levels were statistically significantly reduced in the second half of the cycle (β = −0.09 for AMH^0.3; P < .032).

Individual women’s results for AMH in the early follicular and late luteal phases are shown in Figure 2A, and the maximum and minimum concentrations of AMH for each cycle are shown in Figure 2B. The mean ± standard error of the mean (SEM) difference between maximum and minimum AMH levels was 6.7 ± 1.2 pmol/L, with 31% to 163% changes in AMH levels over the cycle. The intraindividual variance for AMH estimated from a cyclic regression model was 80%, similar to FSH, indicating an approximately 20% interindividual variation. The strongest correlations with AMH were with age (r = −0.38) and day of cycle (r = −0.28) (Spearman correlation test, P < .001, P = .011). The level of AMH was not statistically significantly correlated with the levels of estradiol (r = −0.07), FSH (r = 0.10), or LH (r = 0.18) (all P > .1).

**Variation from Intraindividual Mean**

Within individuals, AMH levels increased from the mean in early follicular phases and decreased in the late luteal phase (Fig. 1D). In the midfollicular phase, the AMH level was typically 20% above the mean (mean ± SEM % = 20.4% ± 9.0%), and in later luteal phases it was almost 20% below the intraindividual mean (mean ± SEM % = −19.0% ± 5.3%). The shifts in the proportional change in AMH between the early follicular phase and middle to late luteal phases of the cycle were statistically significant (P = .004 and P < .001, which were less than the Bonferroni corrected significance level α = .01). There was evidence of a cyclic component in the proportional change in AMH (β = −0.16 for periodic term fitted to day of cycle, P < .001), but the aforementioned phasic model was an improved fit to the data (P = .006, F test for comparison of models).
Crossing Clinical Cutoff Points

The AMH results were assessed throughout the cycle against four cutoffs for definition of reduced ovarian reserve and two for definition of risk of excessive response, as shown in Table 2. For reduced ovarian reserve, 4 or 5 of the 12 women crossed the cutoff, depending on the definition used. Two women crossed the cutoff for being at risk of an excessive response to ovarian stimulation, depending on which day the blood was collected. Using the cutoffs suggested by Nelson et al. (4), AMH levels in over half (7 of 12) the women crossed a clinical cut point over the menstrual cycle, with more than one-third having an altered assessment of ovarian reserve and 2 of 12 having an altered assessment for risk of hyperstimulation.

DISCUSSION

This pilot study was initiated when laboratory staff observed different repeat AMH levels at different phases of the menstrual cycle from a woman who was presenting for fertility treatment at a local clinic. The referring clinician also expressed concern that classification of this patient’s ovarian status could be altered. Collection, processing, and analysis had been performed in the same manner and with the same assay; in collaboration with the fertility clinic, we agreed to further clarify AMH levels throughout the cycle in a group of consecutively presenting women.

This pilot study found clinically significant changes in AMH for day of cycle, phase of cycle, and between follicular and luteal phases of cycle over the spontaneous menstrual cycles of women with at least one AMH measurement indicative of adequate ovarian reserve. The level of AMH was higher in the early follicular phase compared with the late luteal phase and was higher in preovulatory than postovulatory samples. Depending on the criteria used, over half the women crossed cutoffs for assessment of either ovarian reserve or likely excessive response to IVF at different times during the menstrual cycle; however, the small number of women assessed in this study suggests that these findings should be regarded with caution until confirmation with a larger cohort.

### TABLE 2

<table>
<thead>
<tr>
<th>Published cutoff criteria</th>
<th>Adequate ovarian reserve</th>
<th>Clinical category</th>
<th>Risk of ovarian hyperstimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cutoff</td>
<td>No. crossing the cutoff</td>
<td>Cutoff</td>
</tr>
<tr>
<td>Nelson et al. (4)</td>
<td>6.4 pmol/L</td>
<td>5/12</td>
<td>20.4 pmol/L</td>
</tr>
<tr>
<td>Tremellen et al. (9)</td>
<td>8.1 pmol/L</td>
<td>4/12</td>
<td>–</td>
</tr>
<tr>
<td>Friden et al. (8)</td>
<td>8.6 pmol/L</td>
<td>4/12</td>
<td>–</td>
</tr>
<tr>
<td>Nardo et al. (3)</td>
<td>9.4 pmol/L</td>
<td>4/12</td>
<td>36.8 pmol/L</td>
</tr>
</tbody>
</table>

Note: Results in 12 women throughout cycle were assessed in comparison with published cutoffs for adequate ovarian reserve and risk of hyperstimulation as listed, and the number of women crossing cutoffs was recorded. Anti müllerian hormone (AMH) cutoffs have been converted if required to provide values in Beckman Coulter Gen II assay equivalent results (pmol/L).

The difference in median AMH over a cycle was 7.9 pmol/L and the mean change in AMH from minimum to maximum throughout a cycle was 6.7 pmol/L with up to 163% change. The contribution to variance from an individual’s AMH result was 80%, and AMH correlated best with age and next best with day of cycle. The mean percentage deviation in women from their mean AMH level was up to 20% increase in the midfollicular phase and 20% lower in the late luteal phase. However, as the study size was small, these findings need to be confirmed in a larger study.

Nonetheless, our findings are similar to those of Cook et al. (14), Wunder et al. (15), and Sowers et al. (13), who all have documented significant increases in AMH levels in the late follicular and preovulatory phases, compared with lower levels after ovulation or in the early luteal phase. Streuli et al. (16) found a significant decrease in AMH immediately after ovulation but concluded that it was slight and not clinically significant. These findings contrast with earlier studies that found that AMH levels were stable throughout the menstrual cycle (5, 10, 11, 26). Hckenkamp et al. (12) found a possible periovulatory rise in some younger patients with high AMH levels. However, women were compared with a cutoff of 0.5 µg/L (4.4 pmol/L with the AMH Gen II assay), and only 8% of patients crossed this relatively low cutoff for reduced ovarian reserve. Hckenkamp et al. (20) concluded that variability of AMH levels within the menstrual cycle was small. Even with a relatively modest median change in AMH (7.9 pmol/L over cycle), our data illustrate that clinical classification of patients can be altered in over 50% of women with adequate ovarian reserve.

The recent work of Sowers et al. (13) offers some explanation for the discrepancies surrounding AMH during the menstrual cycle. In the Sowers study, two menstrual patterns of AMH were observed, which the investigators denoted as the “aging ovary” and “younger ovary” patterns. The aging ovary pattern had low AMH levels that varied little, in contrast to the younger ovary pattern in which women had higher AMH levels with significant variation during the menstrual cycle, including a rise during the follicular phase. Our study, which included only women with at least one AMH value indicating an adequate ovarian reserve, had findings similar to those of the Sowers group’s younger ovary pattern. We also found AMH levels to be higher during the follicular phase, with a negative trend toward the luteal phase. In the Sowers study, the behavior of the ovary as aging or younger was not related to chronologic age but rather to relative ovarian age, as indicated by higher AMH and inhibin B levels.

Another possible cause of the discrepancies between studies has been the previous availability of two different commercial assays for AMH. Until recently, there have been two major commercial AMH assays, the Diagnostic Systems Laboratories (DSL) kit and the Beckman Coulter assay (16), which give results that were up to 40% different (20). With the recent Beckman Coulter purchase of all patents and the establishment of one assay, the AMH Generation II, better conformity of AMH results is expected (27). Our study used the Beckman Coulter Gen II ELISA assay. Acceptable precision of this assay was demonstrated by Wallace et al. (21). Those investigators also compared the Gen II assay to the Diagnostic Systems Laboratory (DSL) assay and determined that cutoffs of 5 and 15 pmol/L on the DSL assay for prediction of likely poor and excessive responders for fertility therapy corresponded to cutoffs of 6.4 and 20.4 pmol/L using the Gen II assay. Streuli et al. (16) measured their AMH samples using both assays to examine whether assay differences between the DSL kit and the Beckman assay could account for the discrepancies between previous studies. They concluded that the differences found did not result from the use of different assays (16). However, a recent study found the differences between the Beckman Coulter Gen II assay and the DSL assay were contrary to those expected (28).

Previously, AMH levels have been considered to be relatively stable throughout menstrual cycle, including in studies of frozen samples (11), but a recent report (28) raised concern over the stability of AMH by reporting a ~20% rise in frozen samples after 5 days. Others (29) have reported that there is minimal variation in samples frozen at −20°C for 7 days. The most significant variation in AMH appears to occur when whole blood samples are left at room temperature (28, 30). Use of serum tubes with gel separators and prompt separation of serum from cells with storage at 4°C provides reliable stability over more than 5 days (30). As our samples were all collected into serum separator tubes then were promptly centrifuged and frozen, we believe the samples were processed in the optimal manner.

Our findings are in accord with the previous studies (13–15) that found higher early menstrual phase AMH levels. The reasons why AMH may increase in the late follicular compared with the luteal phase are intriguing. Previously it was thought that AMH levels largely reflected the primordial follicular pool and the continuous FSH-independent noncyclic growth of small follicles (5). However, reports have suggested that AMH may reflect the primordial follicular recruitment rates rather than simply primordial follicle number (17, 21). Similarly, Tran et al. (31) suggested that the AMH level may reflect the growing follicular pool that is responsive to gonadotropin. In an interesting report on a case of idiopathic hypogonadotropic hypogonadism, Tran et al. (31) demonstrated that the AMH concentration increased when human menopausal gonadotropin stimulated the growth of FSH-dependent follicles. They concluded that AMH has limitations because it only reflects the growing follicular pool that is responsive to gonadotropins and may not reflect only the underlying primordial pool.

A strength of our study is that the population studied comprised women attending a fertility clinic, which is the setting in which AMH testing has now achieved an accepted role. The women included in our study had samples collected during unstimulated baseline cycles, and the couples’ infertility included a broad range of causes, which reflects general infertility referrals. There are limitations in applying the changes noted in our population to women with normal fertility, so further research is needed to confirm the extent of cycle changes that other investigators (13–15) have noted in women with regular menstruation.

Also, as our study assessed AMH levels in women who had some evidence of adequate ovarian reserve, had spontaneous cycles, and had been referred for fertility therapy, our
findings may not be applicable to other specific groups of women. Our study included a small number of women, so further studies in larger groups that include more young women (who may be more likely to have the variable “younger ovary pattern”) and women with irregular cycles, oligomenorrhea, or amenorrhea may be helpful to clarify and quantify the changes that may be seen in the AMH levels of women presenting for fertility therapy.

CONCLUSIONS

Our pilot study found that over half the women presenting for fertility therapy had AMH levels that crossed clinical decision cut points when AMH was measured throughout spontaneous, unstimulated menstrual cycles. The AMH levels varied over day of cycle and phase of cycle, and they were higher during the follicular phase than the luteal phase, with a negative trend over the menstrual cycle. Although ours was a small study, the classification of women presenting for fertility therapy could change between poor or normal responder in up to a third of women and could change from normal to excessive responder in a smaller proportion of women. As the highest AMH levels were noted during the follicular phase, this may be the optimum time to assess ovarian reserve; however, studies enrolling a greater number of women are needed to confirm these results. In our small study, the classification of a woman’s likely response to IVF depended on the day of cycle that the sample was collected. Further research is needed to clarify the effect of both potential sample instability and biological menstrual variability on the AMH level in women.

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