Published in final edited form as:

Maturitas. 2017 August ; 102: 18–25. doi:10.1016/j.maturitas.2017.04.018.

Anti-Müllerian hormone, follicle stimulating hormone, antral follicle count, and risk of menopause within 5 years

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans and Uniform Requirements for manuscripts submitted to Biomedical journals. Informed consent was obtained for experimentation with human subjects.

Abstract

Objective—To evaluate the ability of concentration of anti-Müllerian hormone (AMH), antral follicle count (AFC), and concentration of follicle stimulating hormone (FSH) to predict the onset of menopause.

Study Design—The Coronary Artery Risk Development in Young Adults Study (CARDIA) Women's Study was an ancillary study to CARDIA, a population-based study of adults aged 18–30 years followed for 3 decades. For this report, participants were women (n=426) who had attended the CARDIA year 15–16 (2000–2001) examination, had at least one ovary, were not pregnant, and underwent serum AMH and FSH measurement and transvaginal ultrasonography in 2002–2003.

Main Outcome Measures—The probability of menopause in 5 years based upon AMH, FSH, and AFC.

Results—The mean age of the women at the time of AMH, FSH, and AFC assessment was 43 years. The cumulative incidence of menopause at 25 years (or follow-up) was 27% (n=426), and the incidence within 5 years was 13% (n=55). Among women aged 45–49 years, undetectable AMH concentrations were associated with a greater than 60% probability of menopause within 5 years, whereas approximately 1/3 of women with no or just one antral follicle experienced menopause within 5 years. Both low and high concentrations of FSH were associated with greater odds of menopause than intermediate concentrations. Models with multiple markers did not improve the prediction of menopause over that afforded by models with single markers.

Conclusion—The ability to predict onset of menopause was improved with any of the three menopausal markers in addition to age. AMH concentrations were more closely associated with menopause than AFC or FSH.

Keywords

menopause; anti-Müllerian hormone; antral follicle count; follicle stimulating hormone

Introduction

1.1. Significance of the prediction of menopause

Menopause is a major milestone of midlife women, because it represents the loss of fertility and the transition to post-reproductive life [1]. While the average age at menopause is approximately 51 years [2], there is significant variation around this estimate. From a clinical perspective, the likely age at menopause is used to guide decision-making regarding contraception [3, 4] assisted reproduction [5], management of menorrhagia [6], and conditions that may fluctuate with menstrual cycle hormonal variations including migraine and premenstrual dysphoric disorder [7]. Thus, markers that can assist in the prediction of menopause are of interest to women and their providers.

1.2. Rationale for the study

Low serum anti-Müllerian hormone (AMH) [8], high serum follicle stimulating hormone (FSH) levels [9], and low antral follicle count (AFC) [10] are associated with the onset of

menopause. However, which of these measures or combination of measures best predicts menopause is not clear. Thus, more data is needed to understand if such markers can improve the prediction of menopause after consideration of age. In addition, more studies are needed which use population-based samples as opposed to selected populations of parous women or women with regular menstrual cycles. Therefore, we examined predictive models for menopause including AMH, FSH, and AFC in a large, multi-center, population-based study of black and white women.

Materials and Methods

2.1. Population and Setting for CARDIA

The Coronary Artery Risk Development in Young Adults (CARDIA) Study and the CARDIA Women's Study have been previously described in detail.[10, 11] Briefly, CARDIA participants aged 18–30 years were recruited from the populations of Birmingham, Chicago, Minneapolis, and northern California, and baseline examinations were performed in 5115 individuals (2788 women) in 1985–1986. The population was balanced according to age, sex, race and educational attainment at each site. The CARDIA Women's Study was an ancillary study to CARDIA designed to examine the associations between androgens, polycystic ovaries, and associated clinical features with subclinical atherosclerosis. Eligibility criteria included attendance at the year 15 CARDIA examination (2001–2002), presence of at least one ovary, and lack of current pregnancy. The CARDIA Women's Study included 1163 participants, and the original protocol did not include AFC. However, with the publication of the Rotterdam criteria for polycystic ovary syndrome in 2003 [12], the protocol was altered to include AFC assessments. Thus, of the 1163 participants, 705 underwent AFC assessments. An institutional review board at each site approved all study procedures, and written informed consent was obtained from study participants prior to assessments.

2.1. Population for the current substudy

For the current report, we included women in the CARDIA Women's Study. Eligible participants had to have undergone serum measurements of AMH and FSH as well as transvaginal ultrasonography for AFC at the 2002–2003 exam. Participants were excluded if they had bilateral oophorectomy, were pregnant, or did not attend the parent CARDIA exam in 2001–2002. For the current report, women with hysterectomy at any time were excluded, leaving 426 women. As compared with the final sample, excluded women were more likely to have been black (58% vs. 44%, $p<0.01$) and to have had slightly higher FSH concentrations (7.2 IU/L vs. 6.4 IU/L, $p<0.01$) but otherwise had similar age, AMH concentrations, and similar values for smoking, body mass index (BMI), and oral contraceptive pill (OCP) use.

2.2. Outcome

The primary outcome was menopause within the 5 years after ovarian reserve assessment. Participants completed a self-administered questionnaire during the CARDIA 2005–2006 (Year 20) and 2010–2011 (Year 25) examinations which enquired after cessation of menses and whether cessation occurred naturally, surgically, or through other means such as

chemotherapy [10]. In a validation study, of the 18 women who reported that their menses had ceased at the 2002–2003 visit, 78% had FSH levels ≥ 40 IU/L, and among 202 women who reported both their age and calendar date of their final menstrual period, 94% reported a date which matched within one year of their self-reported age at menopause. At each visit, standardized questionnaires and examinations included assessment of lifestyle behaviors, including smoking and oral contraceptive use, information on regularity of menses, and anthropometrics. BMI was calculated in kg/m^2 .

2.3. FSH, AMH, AFC

Blood samples were drawn on the day of the transvaginal ultrasound. Visits were timed to the follicular phase (days 1–10 of the menstrual cycle) among regularly cycling women; women using OCPs or women with irregular menstrual cycles were scheduled for a visit when convenient. Within one hour of blood draw, samples were processed into aliquots and frozen at -70°C . FSH was measured in serum using a sequential two-step immunoenzymatic “sandwich” assay (Beckman Coulter, Brea, CA) by the University of Alabama Birmingham Diagnostic Laboratory. The intra-assay coefficients of variation ranged from 3.8 – 5.4% and interassay coefficients of variation ranged from 3.9 – 4.2%. In 2014, AMH was measured in stored samples from the 2002–3 visit using Ansh Laboratories (Webster, TX) ultra-sensitive AMH ELISA platform at the clinical reference laboratory, ReproSource, (Woburn, MA). The clinical lower limit of detection was 0.02 ng/dl; intra-assay coefficients of variation ranged between 3–7% and interassay coefficients of variation ranged between 5–10%.

Transvaginal ultrasound was performed by American Registry for Diagnostic Medical Sonography certified sonographers. Thirteen sonographers (a range of 2–5 sonographers per CARDIA field center) performed transvaginal ultrasounds. A 5–7.5 MHz vaginal probe was used to search for each ovary. If no ovary was found after 2 minutes, iliac vessels were photographed. When an ovary was found, antral follicles (follicles measuring 2–10 mm) were counted. AFC was defined as the total number of follicles on both ovaries. The CARDIA Women’s Study included a quality assurance protocol for ultrasonography. For transvaginal ultrasound, data from a repeat transvaginal ultrasound by a sonographer was available for a subset of 28 participants. For AFC ≤ 4 vs. > 4 , interrater reliability was high ($\kappa=0.85$) between the repeat ultrasound and the original ultrasound.

2.4. Statistical Analysis

2.4.1. Classification of age, AMH, AFC, and FSH—Age, AMH, AFC, and FSH were treated as categorical variables when calculating descriptive statistics and then as continuous variables when examining the relationship with menopause onset at 5 years. Currently, there is no consensus with these variables as to which categories should be used to predict age at menopause. Therefore, for the descriptive analyses, we used cutpoints commonly used in clinical practice and which maximized the sensitivity or specificity of that particular measure. For AMH, cutpoints of <0.09 ng/dl (the lower limit of detection of the AMH assay) and ≥ 2.0 ng/dl were used. For AFC, cutpoints of < 1 follicle vs. ≥ 10 follicles were examined. Although an FSH concentration of 40 IU/L is commonly considered consistent with postmenopause [13], values this high were rare in this premenopausal population ($n=6$),

consistent with controversy as to which FSH value should be used in the premenopausal stage to predict age at menopause. Thus, extremes of FSH were defined as the lowest quartile (< 5.3 IU/L) vs. the uppermost quartile (> 13.0 IU/L). Spearman's correlation was used to estimate the associations among continuously measured AMH, AFC, and FSH.

2.4.2. Multivariable models—Multivariable logistic regression was used to predict the cumulative probability of menopause within 5 years of measurement. Age, AMH, AFC, and FSH were modeled as continuous covariates using restricted cubic splines to allow for non-linear associations. We considered 8 prediction models and compared model fit using the C-Index (area under the receiver operator characteristic curve), Akaike Information Criterion (AIC) [14], and likelihood ratio tests indicating whether goodness-of-fit differed between models. Since age is known to be a key predictor of menopause, an “age only” model was considered our baseline model for comparison. Bootstrap cross validation (150 replications) was used to provide corrected indices of model fit. Initially, we used Cox regression models to examine the association between each marker and incident natural menopause. However, the proportional hazards assumption was violated, therefore, we used logistic regression models which focused on the odds of menopause within 5 years. Odds were modeled flexibly using regression splines and translated into probability = odds/(1+odds) for figure plotting.

2.4.3. Sensitivity analyses—To make the analyses applicable to a general population, our main analyses included all women, including women using OCPs and women with irregular menses. However, we conducted multiple sensitivity analyses focusing on the following subpopulations: 1) women not using OCPs, 2) women with regular menses who had marker assessment during days 1–10 of their menstrual cycle, and 3) women without a single dominant antral follicle (>20 mm). All statistical analyses were performed using R statistical software (version 3.3.0).

Results

3.1. Participant characteristics

Participant characteristics are shown in Table 1. At the year 15–16 examination, women were approximately 43 years of age and 44% were black. Approximately 20% smoked, and the majority was overweight or obese. While most women had regular menstrual cycles, 15% of women were not able to predict their period for half of their cycles. Twenty-seven percent ($n=117$) had achieved natural menopause by year 25 follow-up, and approximately 55 women achieved natural menopause within 5 years of their AMH, AFC, or FSH measure.

3.2. Distribution and correlation of AMH, AFC, and FSH

Continuous values of AMH and AFC were positively correlated ($r=0.51$, $p<0.01$), whereas FSH was negatively and similarly correlated with both AMH and AFC ($r=-0.22$ and $r=-0.20$ respectively, both $p<0.01$). Table 2 shows the proportion of women with low and high AFC and FSH values by category of AMH. The middle quartiles of menopausal measures were combined for simplicity of presentation. Women with undetectable AMH concentrations were more likely to have low follicle counts and elevated FSH

concentrations, and women with AMH ≥ 2.0 ng/dl were more likely to have high follicle counts and lower FSH concentrations ($p < 0.01$). Similarly, women with low follicle counts were more likely to have high FSH concentrations and women with high follicle counts were more likely to have low FSH concentrations ($p < 0.01$).

3.3. AMH, AFC, FSH and cumulative incidence of menopause within 5 years

Figure 1 shows the percentages of women who were menopausal within 5 years of their AMH, AFC, and FSH measure. Few women 34–39 years of age experienced their final menstrual period in the next 5 years, and thus AMH, AFC, and FSH could not distinguish which women would experience menopause in this age category. Regardless of age, no women with AMH concentrations ≥ 2.0 ng/dl experienced menopause within 5 years (Figure 1a). Among women aged 45–49 years, 60% of women with undetectable AMH concentrations experienced menopause within 5 years. Women with high AFC were less likely to experience menopause, although approximately 1/5 of women with high follicle counts i.e. ≥ 10 still underwent menopause. Among women with only 0–1 follicles, approximately 1/3 underwent menopause within 5 years (Figure 1b). The relationship between FSH and probability of menopause was U-shaped, where both high and low FSH were associated with a higher incidence of menopause: although a greater proportion of women with FSH ≥ 13.0 IU/L experienced menopause within 5 years than women with lower concentrations, approximately 45% of women in the lowest quartile of AMH underwent menopause within 5 years, limiting the utility of FSH for distinguishing between women at higher vs. lower risk. This pattern of results for AMH, AFC, and FSH was similar among women not using OCPs, women menstruating regularly and with marker measurement during their follicular phase, and women without a single dominant follicle (results not shown).

3.4. Odds of menopause by AMH, AFC, and FSH in multivariable models

Figure 2 shows the estimates of the probability of menopause within the next 5 years from logistic regression models by age at AMH measurement and AMH concentration. No matter how low the AMH concentration, the probability of menopause was low for women in their thirties (bottom line on Figure 2). Similarly, no matter how high the AMH concentration, the probability of menopause was high for women who were 50 years of age (top line on Figure 2). The probability of menopause for women over 45 years of age was high if their AMH levels were undetectable. The relationship between age, AMH concentrations, and probability of menopause was logarithmic, such that even slightly higher AMH in the range between undetectable concentrations and 0.5 ng/dl had a significant impact on menopause probability. Estimates of probability for women not using OCPs (Appendix Figure 1) and among women with regular menses and follicular phase marker assessment (Appendix Figure 2) also showed a logarithmic relationship between AMH concentrations and probability of menopause within 5 years. OCP use ($p = 0.91$) did not modify probability curves; regular menses had borderline significance for modifying the curves ($p = 0.07$), but predicted probabilities were close to 0 and estimates of confidence were wide.

Table 3 shows the odds of menopause within 5 years for each menopausal marker. Given that the odds ratios changed depending upon women's ages and with the range of

menopausal markers, the odds ratios shown focus on the group of women aged 45–49 years, with AMH levels between 0.09 and 0.5 ng/dl, with FSH <14 IU/L, and with AFC between 0–10 follicles. After adjustment for age, AMH was the only menopausal marker significantly associated with odds of menopause. Table 3 also shows estimates of model fit for logistic regression models examining the odds of menopause within 5 years. Models containing both age and AMH had the highest C-index and lowest AIC (consistent with better model quality), followed by models including age and other markers. When compared to models including age alone, model fit was improved with addition of any menopausal marker or combination of markers. When compared to models containing age and AMH, addition of FSH and/or AFC did not lead to improved model fit. Further adjustment for covariates including covariates in Table 1 did not change this pattern (results not shown). Again, these patterns were similar in sensitivity analyses among women not using OCPs, women with regular menses and follicular phase measurement, and women without a single dominant follicle (Appendix Table 1).

Discussion

4.1. Summary of findings: AMH

In a population-based cohort, measurement of AMH best improved the prediction of menopause probability within 5 years compared to age alone and compared to other menopausal markers. Due to the low probability of menopause within 5 years for younger women, ovarian reserve markers had higher predictive value for older women. AMH concentrations > 2.0 ng/dl were associated with minimal probability of menopause within the next 5 years regardless of a woman's age, and conversely, AMH concentrations below detection level were associated with a greater than 60% probability of menopause among women aged 45 years and older. Of note, even small changes in AMH above undetectable levels correlated with large decreases in probability of menopause, particularly among women aged greater than 45 years of age.

4.2. Summary of findings: AFC and FSH

Although higher AFC was associated with a lower proportion of women experiencing menopause than a lower AFC, only about one-third of women aged 45–49 years with 0–1 follicles underwent menopause, suggesting that low AFC counts had weaker predictive value than low AMH for the onset of menopause. While models including AFC improved menopause prediction compared to models with age alone, AFC values themselves were not significantly associated with odds of menopause. While FSH concentrations improved the prediction of menopause after consideration of age, the relationship between FSH and the onset of menopause was U-shaped and thus difficult to use for estimation of menopause probability. Approximately 45% and 65% of women aged 45–49 years in the lowest and highest quartile of FSH, respectively, experienced menopause within 5 years.

4.3. Previous studies reporting upon AMH, AFC, and FSH for the prediction of menopause

Previous reports, including those by our group as well as others, have noted that measures of ovarian reserve can predict age at menopause [8, 10, 15–20]. However, implementation in the clinical setting has been limited by several factors. First, the detection limits of AMH

varied widely with the first generations of assays [21], making cutpoints difficult to determine. Second, the logistical difficulties inherent in assessing AFC in a uniform manner across centers [22] and the invasiveness of transvaginal ultrasound have limited widespread use of AFC. Third, the fluctuation of FSH between extreme elevations and depressions in the perimenopause [23], even when assessed in the follicular phase of the menstrual cycle and in the absence of estrogen use, have limited the use of FSH for these purposes. Finally, the strength of the association between markers and age menopause weakens over time; specifically, the longer the time to menopause, the weaker the association between menopausal marker and age at menopause [15]. Such changes in the strength of association with time impair the value of these markers for menopause prediction.

4.4. Methodologic strengths of the current report

However, our current report adds to the previous literature in several respects. First, the present report of 426 women is the largest containing more than a single marker of ovarian reserve. Thus, we were able to compare the associations between several markers of ovarian reserve and time to menopause. Second, by examining the proportion of women who reached menopause within a specific time frame, we were able to present results in a manner which acknowledges that risk of menopause changes over time. Along these lines, we present the odds of menopause within 5 years for measures consistent with high ovarian reserve as well as for low ovarian reserve. Information is displayed graphically for AMH to aid ease of interpretation. Third, the cohort included women who were drawn from a population-based sample, whereas previous cohorts focused upon women with regular menstrual cycles, women undergoing infertility evaluations, and women not using OCPs, interfering with the interpretation of results from women who did not fit these criteria. Similarly, approximately half of the population in our report was African-American, an important characteristic to consider given that the CARDIA study [8] and at least one other report have noted racial/ethnic differences in AMH concentrations between blacks and whites [24]. Finally, our study addresses several of the limitations of ovarian reserve measurements mentioned in the previous paragraph, including imprecise and relatively less sensitive AMH assays; AFC estimates generated from single-center reports; and the inaccuracy of risk models which assume uniform risks over time.

4.5. Clinical relevance

Although historically used to guide assisted reproductive therapy, markers of ovarian reserve have potential application to the larger population of women in perimenopause. Such symptoms include menorrhagia, which in the Study of Women's Health Across the Nation affected almost one-third of late reproductive-stage women [25] and which lowers quality of life [26]. The choice of therapy for such symptoms, including intrauterine device insertion, endometrial ablation, hysterectomy, and systemic hormone and anti-platelet therapy, are informed by expected length of time with symptoms, i.e. time to final menstrual period as well as individual factors. Such symptoms also include those associated with the hormone fluctuation characterizing the menopause, such as menstrual migraines, which affect over one in 10 perimenopausal women [27] and may be treated with estrogen therapy. However, estrogen therapy obscures the menstrual cycle, and currently therapy must be punctuated by trials off of medication, which in turn are associated with exacerbation of symptoms. Finally,

ovarian reserve markers may be of use in the prediction of conditions associated with the postmenopause, for which current risk markers are limited. For example, bone density testing for the identification of women with osteopenia is not recommended until 65 years of age [28], although such guidelines have the potential for missing the subset of women with markedly accelerated bone loss preceding the final menstrual period [29]. Thus, estimates of the length of time to menopause are of value aside from estimates of fecundity.

4.6. Limitations

Limitations of this report include lack of more precise estimates of the proportion of women reaching their final menstrual period, i.e. within 1 year or 6 months. Such estimates would require more precise methods of determining the age at the final menstrual period rather than the self-report employed in CARDIA, as well as a larger population-based sample that would allow for estimation before and after covariates such as BMI and genetic variation that also determines ovarian reserve [16]. Such a cohort is unlikely to be amassed given the length of follow-up time required as well as the invasive nature of transvaginal ultrasounds and difficulty of timing FSH measures to the menstrual cycle. We may have underestimated the predictive capability of FSH, as this marker fluctuates throughout the menstrual cycle and is typically measured on the 3rd day of menstruation. However, for those women with irregular menstrual cycles or who use sex steroids, measurement on day 3 is difficult. In addition, the non-linear relationship between FSH and time to menopause limits its use for menopause prediction. The length of follow-up did not allow comparison of markers for women of younger reproductive age or reproductive ages greater than 49 years, and our results do not apply to these age extremes. Finally, estimates of time to menopause used only a single measure of AMH, and it is possible that AMH levels decline at different rates between women [30].

4.7. Conclusions

We conclude that among late reproductive-age women, AMH concentrations can predict the odds of menopause within the next 5 years, and specifically, AMH greater than 2.0 ng/dl is associated with an extremely low probability of menopause whereas undetectable concentrations predict a higher risk of menopause among women in their forties. These estimates may be of value to women and their physicians in the management of reproductive disorders. Other markers of ovarian reserve may also be useful even after consideration of a woman's age. However, due to non-linearity in FSH concentrations with probability of menopause, need for timing to the follicular phase of the menstrual cycle, and the necessity of transvaginal ultrasound for the assessment of AFC, FSH and AFC may be more difficult to apply to menopause prediction in the clinical setting. Future investigations should also explore the value of AMH in risk prediction for disorders associated with menopause, such as osteopenia and atherosclerosis, as well as the management of conditions associated with the management of menorrhagia in the perimenopause.

Acknowledgments

Funding

This work was supported by the National Institutes of Health (HHSN268201300025C, HHSN268201300026C, HHSN268201300027C, HHSN2682013000- 28C, HHSN268201300029C, HHSN268200900041C, AG005, R01-HL-065611, K23-HL-87114, R03-HL-135453, and DP3-DK-098129).

Abbreviations

AFC	antral follicle count
AMH	anti-Müllerian hormone
BMI	body mass index
CARDIA	Coronary Artery <u>Risk Development in Young Adults</u> Study
ELISA	enzyme linked immunosorbent assay
FSH	follicle stimulating hormone
OCP	oral contraceptive pill

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Appendix

Appendix Figure 1.
Probability of menopause within 5 years for women with regular menses.

Appendix Figure 2.

Probability of menopause within 5 years for women who are not using oral contraceptives.

Ancillary Table 3

Comparisons of model fit for models containing age and menopausal markers (anti-Müllerian hormone or AMH, antral follicle count or AFC, follicle stimulating hormone or FSH).

	C-statistic for models with all women (n=426)	C-statistic for models in women not using estrogen. (n=359)	C-statistic for models with women with regular menses only. (n=327)	C-statistic for models excluding any woman with a dominant follicle. (n=306)
Age alone	0.83	0.82	0.81	0.86
Age and AMH	0.91	0.91	0.91	0.94
Age and AFC	0.84	0.84	0.82	0.86
Age and FSH	0.86	0.86	0.84	0.89
Age, AMH, FSH	0.91	0.92	0.91	0.94
Age, FSH, AFC	0.86	0.87	0.84	0.89
Age, AMH, AFC	0.91	0.91	0.91	0.95
Age, AMH, FSH, AFC	0.91	0.92	0.91	0.95

Highlights

- Using data from a population-based study of adults, we evaluated the ability of several measures of ovarian age to predict the onset of menopause.
- Among women aged 45–49 years, those with undetectable anti-Müllerian hormone concentrations had a 60% probability of menopause within 5 years.
- Among women aged 45–49 years, approximately one-third of women with no or just one antral follicle underwent menopause within 5 years.
- The concentration of follicle stimulating hormone had a non-linear relationship with the likelihood of menopause within 5 years.

Figure 1.
Percentage of women who achieve menopause within 5 years after assessment of AMH (Panel A), AFC (Panel B), and FSH (Panel C).

Figure 2. Probability of menopause within 5 years by age and anti-Müllerian hormone (AMH) concentration based on logistic regression models. Lower limit of AMH detection is 0.09 ng/dl.

Table 1

Characteristics of women at assessment of anti-Müllerian hormone (AMH), follicle stimulating hormone (FSH), and antral follicle count (AFC) (n=426), at the year 15–16 examination. Median and interquartile range or n (%) shown.

Age at year 15 exam (years)	43 (39–45)
White (n,%)	240 (56%)
Education (years)	15 (13–16)
Body mass index (kg/m ²)	28 (24–34)
Current oral contraceptive pill use (n, %)	67 (16%)
Current cigarette use (n,%)	83 (19%)
Regular menses (n,%)	364 (85%)
AMH (ng/dl)	0.77 (0.22, 2.00)
AMH categories (n, %)	
Undetectable	57 (13%)
0.09–1.99 ng/dl	262 (62%)
2.0 ng/dl	107 (25%)
AFC (number of follicles)	5 (2–9)
AFC categories (n,%)	
0–1 follicles	91 (21%)
2–9 follicles	235 (55%)
10 follicles	100 (23%)
FSH (IU/L)	6.4 (4.6, 9.1)
FSH categories (n,%)	
0–5.39 IU/L	103 (24%)
5.4–12.9 IU/L	259 (61%)
13.0 IU/L	64 (15%)

Table 2

Number and proportion of women by anti-Müllerian hormone (AMH), antral follicle count (AFC), and follicle stimulating hormone (FSH) categories.

	AMH (ng/dl)		
	<u>Undetectable</u>	<u>0.09–1.9</u>	<u>> 2.0</u>
<u>AFC (follicle number)</u>			
0–1	30 (53%)	54 (21%)	7 (7%)
2–10	27 (47%)	173 (66%)	35 (33%)
10	0	35 (13%)	65 (61%)
	AMH (ng/dl)		
<u>FSH (IU/L)</u>	<u>Undetectable</u>	<u>0.09–1.9</u>	<u>> 2.0</u>
0–5.39	5 (9%)	63 (24%)	35 (33%)
5.4–12.9	22 (38%)	165 (63%)	72 (67%)
13.0	30 (52%)	34 (13%)	0
	AFC (follicle number)		
<u>FSH (IU/L)</u>	<u>0–1</u>	<u>2–9</u>	<u>≥10</u>
0–5.39	22 (24%)	58 (25%)	23 (23%)
5.4–12.9	40 (44%)	144 (61%)	75 (75%)
13.0	29 (32%)	33 (14%)	2 (2%)

Table 3

Comparisons of model fit for models containing age and menopausal markers (anti-Müllerian hormone (AMH), antral follicle count (AFC), and follicle stimulating hormone (FSH). Odds ratios (95% confidence intervals) for age, AMH, AFC, and FSH shown; bold type indicates statistical significance at $p < 0.05$. *

	Odds ratios (95% confidence intervals)	C-statistic	Akaike Information Criterion (AIC)	Likelihood ratio test vs. models with age alone	Likelihood ratio test vs. models with age and AMH
Age alone		0.83	254.9	-	-
Age (years)	7.4 (4.1, 13.6)				
Age and AMH		0.91	199.4	<0.001	
Age (years)	4.1 (2.1, 7.9)				
AMH (ng/dl)	0.13 (0.05, 0.34)				
Age and AFC		0.84	250.1	0.009	
Age (years)	6.5 (3.5, 12.0)				
AFC (number)	0.33 (0.13, 8.2)				
Age and FSH		0.85	239.4	<0.001	0.077
Age (years)	6.1 (3.3, 11.3)				
FSH (IU/L)	0.79 (0.18, 3.6)				
Age, AMH, FSH		0.92	198.3	<0.001	
Age (years)	3.9 (2.0, 7.8)				
AMH (ng/dl)	0.18 (0.07, 0.43)				
FSH (IU/L)	0.47 (0.10, 2.3)				
Age, FSH, AFC		0.86	238.5	<0.001	0.275
Age (years)	5.7 (3.0, 10.6)				
AFC (number)	0.47 (0.19, 1.2)				
FSH (IU/L)	0.77 (0.17, 3.4)				
Age, AMH, AFC		0.91	200.2	<0.001	
Age (years)	4.1 (2.1, 8.0)				
AMH (ng/dl)	0.12 (0.04, 0.31)				

	Odds ratios (95% confidence intervals)	C-statistic	Akaike Information Criterion (AIC)	Likelihood ratio test vs. models with age alone	Likelihood ratio test vs. models with age and AMH
AFC (number)	2.0 (0.57, 6.9)				
Age, AMH, FSH, AFC		0.91	198.3	<0.001	0.070
Age (years)	3.9 (2.0, 7.8)				
AMH (ng/dl)	0.15 (0.06, 0.39)				
FSH (IU/L)	0.44 (0.09, 2.2)				
AFC (number)	2.4 (0.69, 8.6)				

* For estimation of odds ratios, model assumptions are shown for women aged 45–49 years, with AFC 0–10 follicles, FSH 0–13.9 IU/L, and AMH 0.09–0.50 ng/dl.