Anti-müllerian hormone is a promising predictor for the occurrence of the menopausal transition

Ilse A.J. van Rooij, PhD,[1] Isolde den Tonkelaar, PhD,[2] Frank J.M. Broekmans, PhD,[1] Caspar W.N. Looman, MSc,[3] Gabrielle J. Scheffer, PhD,[1] Frank H. de Jong, PhD,[4] Axel P.N. Themmen, PhD,[4] and Egbert R. te Velde, PhD[1]

ABSTRACT

Objective: Age at menopause and age at the start of the preceding period of cycle irregularity (menopausal transition) show considerable individual variation. In this study we explored several markers for their ability to predict the occurrence of the transition to menopause.

Design: A group of 81 normal women between 25 and 46 years of age visited the clinic two times (at T_1 and T_2) with an average interval of 4 years. All had a regular menstrual cycle pattern at T_1. At T_1, anti-müllerian hormone (AMH), follicle-stimulating hormone (FSH), inhibin B and estradiol (E_2) were measured, and an antral follicle count (AFC) was made during the early follicular phase. At T_2, information regarding cycle length and variability was obtained. Menopause transition was defined as a mean cycle length of less than 21 days or more than 35 days or as a mean cycle length of 21 to 35 days, but with the next cycle not predictable within 7 days during the last half year. A logistic regression analysis was performed, with the outcome measure as menopause transition. The area under the receiver operating curve (ROC_{AUC}) was calculated as a measure of predictive accuracy.

Results: In 14 volunteers, the cycle had become irregular at T_2. Compared with women with a regular cycle at T_2, these women were significantly older (median 44.7 vs 39.8 y, $P < 0.001$) and differed significantly in AFC, AMH, FSH, and inhibin B levels assessed at T_1. All parameters with the exception of E_2 were significantly associated with the occurrence of cycle irregularity; AMH, AFC, and age had the highest predictive accuracy (ROC_{AUC} 0.87, 0.80, and 0.82, respectively). After adjusting for age, only AMH and inhibin B were significantly associated with cycle irregularity. Inclusion of inhibin B and age to AMH in a multivariable model improved the predictive accuracy (ROC_{AUC} 0.92).

Conclusions: The novel marker AMH is a promising predictor for the occurrence of menopausal transition within 4 years. Adding inhibin B improved the prediction. Therefore, AMH alone or in combination with inhibin B may well prove a useful indicator for the reproductive status of an individual woman.

Key Words: Anti-müllerian hormone – Inhibin B – Follicle-stimulating hormone – Menopause transition – Reproductive stage – Cycle irregularity.

Menopause, defined as the final menstrual period, marks the end of the female reproductive life span. This event occurs at a median age of about 51, but varies from 40 to 60 years.[1,2] Irrespective of age at menopause, cycle irregularity occurs about 4 to 5 years before the final menstrual period,[3] whereas the end of fertility

commences at a median age of 41 years with a distribution similar to that of age at the last menstrual period.[4,5] We assume that these events follow a time sequence with more or less fixed intervals, with the end of fertility occurring circa 10 years before the age at menopause.[2]

The decline of the number of follicles seems to dictate these reproductive events. During the fetal period, the ovaries are endowed with a limited stock of follicles that serve the rest of a woman's reproductive life. At puberty some 300,000 follicles are left, and this number will decline with age, with a more rapid decrease from 37 years on,[6] corresponding with a more pronounced decline in fecundity.[7,8] When follicle numbers fall below a critical threshold of a few thousand, the menstrual cycle pattern becomes irregular.[9] At menopause, fewer than 1,000 follicles are left.[6]

Because of the variation as described in the first paragraph, chronological age is a relatively poor indicator of the stage of reproductive aging in individual women. Recently, a staging system for reproductive aging was proposed (STRAW) with the aim to identify which level of reproductive decline an individual woman has reached.[10] Menopause was used as a key event, preceded by the so-called menopausal transition, when cycles become irregular. Before the start of the menopausal transition, a stage characterized by regular cycles and elevated follicle-stimulating hormone (FSH) levels was discerned, called the late reproductive phase. FSH levels rise on average after the age of 35 years,[11] but younger women also may have higher FSH levels, which are physiological and may be related to receptor polymorphisms and not to ovarian aging.[12,13] Furthermore, the most substantial changes in FSH levels occur during the menopausal transition, when cycles are already irregular.[14]

In addition to FSH, other tests reflecting ovarian reserve are available, which could be of use in assessing an individual's reproductive status. Inhibin B is a product of granulosa cells of the recruited antral follicle cohort and is thought to decline before the FSH rise and, therefore, to be an earlier marker.[15] The number of antral follicles, or antral follicle count (AFC), as assessed by ultrasonography, is likely to represent the size of the primordial follicle pool.[16] Anti-müllerian hormone (AMH), which is produced by primary, secondary, and antral follicles, also has proven to be a promising marker of reproductive decline.[17,18]

In the current study we explored the ability of these endocrine and ultrasound markers to predict the occurrence of the menopausal transition within about 4 years in a group of women with normal fertility in the past.

METHODS

Participants

For an ongoing longitudinal study on ovarian function, healthy female volunteers were recruited in 1996 and 1997. This study is part of that longitudinal study. Women could be included if they were between 25 and 46 years of age and had a regular menstrual cycle of 21 to 35 days, with the next cycle predictable within 7 days. All women had a proven natural fertility, which was defined as having established at least one pregnancy within 1 year after stopping contraceptives, resulting in a normal delivery at term. If a woman used hormonal contraceptives, this had to be stopped at least 3 months before the start of the study. Exclusion criteria were ovarian surgery or ovarian abnormalities. The Institutional Review Board approved the study, and written informed consent was obtained from all participants. The volunteers received monetary compensation for participating.

Of the 162 volunteers initially included, 82 women continued in the longitudinal part of the study. These women were older than were the 80 women who did not continue. Reasons for not participating in the longitudinal part were: wish to continue hormonal contraception (n = 10), pregnancy or lactation (n = 3), disease requiring intervention (n = 3), lack of time (n = 8), or having moved (n = 37). For 19 volunteers, the reason for not participating was unknown.

Study design

Volunteers visited the clinic for the first time (T_1) during the early follicular phase of the menstrual cycle (on cycle day 2, 3, or 4), for an assessment of the number of antral follicles by transvaginal ultrasonography (Toshiba Capasee SSA-220A, Toshiba Medical Systems Europe BV, Zoetermeer, The Netherlands)[16] and to provide blood samples. Serum and plasma were separated and stored at −20°C for later estimation of levels of AMH, FSH, inhibin B, and estradiol (E_2).

The women returned on average 4 (range 3-5) years later for their second visit (T_2). During the T_2 visit, information regarding the menstrual pattern between T_1 and T_2 was obtained. Participants were questioned on mean cycle length and the variability of cycle length.

Outcome measure

No uniform definition for the transition to menopause (cycle irregularity) is available, but recently some definitions based upon increasing variability in cycle patterns have been proposed.[10,19] We defined the

outcome measure of menopausal transition in two ways: (A) Mean cycle length is between 21 and 35 days, but in the last half year, the next menstrual period is not predictable within 7 days; and (B) Mean cycle length is less than 21 or more than 35 days during the last half year or longer. To be able to determine cycle irregularity, all women should have stopped hormonal contraception at least 3 months before the visit at T_2. None of the volunteers used hormone therapy (HT) for menopause complaints. Two women were using hormonal contraceptives, and when they stopped, both had a regular cycle pattern in the subsequent half year.

Assays

Levels of FSH and E_2 were measured in plasma with the AxSYM immunoanalyzer (Abbott Laboratories, Abbott Park, IL, USA). The World Health Organization Second International Reference Preparation for human FSH (78/549) was used as a standard in the FSH assay. For FSH, interassay coefficients of variation were found to be 5.7%, 5.7%, and 7.8% at the levels of 5, 26 and 79 IU/L, respectively (n = 80). The E_2 assay has been standardized to gas chromatography/mass spectrometry. Interassay coefficients of variation of the E_2 assay at 185, 330, and 1152 pmol/L were 12.9%, 7.8%, and 5.1% (n = 80), respectively. Serum inhibin B levels were measured using an immuno-enzymometric assay (Serotec, Oxford, United Kingdom) as described by Groome et al.[20] Interassay coefficients of variation were 6.6% and 6.0% at 110 and 770 ng/L, respectively. An ultrasensitive immunoenzymometric assay (Immunotech-Coulter, Marseille, France) was used for the estimation of AMH.[21] The limit of detection (defined as blank + 3SD of blank) was 0.05 μg/L. Intra- and interassay coefficients of variation were less than 5% and less than 8%, respectively.

Methods of statistical analysis

Data were analyzed with SPSS 10.1 (SPSS Inc., Chicago, IL) and with GLIM (Generalized Linear Interactive Modeling 4; NAG, Oxford, UK). Because the results of the ovarian reserve tests at T_1 were not normally distributed, values are presented as median with 10th to 90th percentiles. For comparisons of ovarian reserve tests performed at T_1 between women with a regular cycle at T_2 and women with an irregular cycle at T_2, the Mann-Whitney test and Fisher exact test were used. To assess the relationships between ovarian reserve tests and age at T_1, Spearman correlation coefficients were calculated.

Univariable and multivariable logistic regression analysis were used to assess the relation between ovar-

TABLE 1. *Comparisons of age and ovarian reserve tests between women with a regular cycle and with an irregular cycle circa 4 years later*

Variables[a]	Regular cycle at T_2 (n = 67)	Irregular cycle at T_2 (n = 14)	P[b]
Age (y)	39.8 (32.0-45.0)	44.7 (39.0-46.5)	<0.001
AMH (μg/L)	1.7 (0.2-3.4)	0.3 (<0.05-1.0)	<0.001
AFC (n)	7.0 (2.8-17.2)	4.0 (1.5-8.0)	<0.001
FSH (IU/L)	7.6 (4.2-11.4)	10.4 (5.7-24.6)	0.01
Inhibin B (ng/L)	116 (28-163)	43 (4-126)	0.002
E_2 (pmol/L)	178 (100-318)	197 (87-537)	0.55

Values are median (10th-90th percentiles). T_2, second visit; T_1, first visit; AMH, anti-müllerian hormone; AFC, antral follicle count, FSH, follicle-stimulating hormone; E_2, estradiol.
[a]Assessed at T_1
[b]Mann-Whitney test

ian reserve markers measured at T_1 and the occurrence of the menopausal transition in the follow-up period of, on average, 4 years. For the multiple analysis, a forward selection with P less than 0.05 for entry was applied. The area under the receiver operating characteristics curve (ROC_{AUC}) was calculated to assess the ability to discriminate between women whose cycle pattern would become irregular and women whose cycle pattern would remain regular.[22] The ROC_{AUC} may vary between 0.5 (no discriminative power) and 1.0 (perfect discrimination). A bootstrapping sampling procedure was applied in which the selection of the variables was repeated 1,000 times after creating 1,000 new data sets (bootstrapping replicas) by drawing cases with replacement from the original data. The probability that a variable was selected in the stepwise procedure was estimated by this procedure.

RESULTS

Because from one woman no AMH value could be determined due to an insufficient amount of serum, data of 81 women could be analyzed. In 14 volunteers, the cycle became irregular between T_1 and T_2. Five women did not have a menstrual period during the 6 months preceding the second visit, five had more than two skipped cycles within the last half year, two had skipped at least one cycle in the last half year, and the other two women could not predict the subsequent cycle with a precision of less than 7 days. The median interval between T_1 and T_2 was 3.9 years, ranging from 3.0 to 4.9 years.

Age and ovarian reserve tests assessed at T_1 were compared between women with a regular cycle and with an irregular cycle circa 4 years later (T_2) (Table 1). Women with an irregular cycle at T_2 were significantly older at T_1 than women still regular at T_2. They also had

TABLE 2. *Correlation between ovarian reserve markers and age assessed at T_1*

	Age	AMH	AFC	FSH	Inhibin B
AMH	−0.66 ($P < 0.001$)				
AFC	−0.74 ($P < 0.001$)	0.67 ($P < 0.001$)			
FSH	0.35 ($P = 0.02$)	−0.44 ($P < 0.001$)	−0.33 ($P = 0.003$)		
Inhibin B	−0.05 ($P = 0.65$)	0.13 ($P = 0.24$)	0.20 ($P = 0.08$)	−0.09 ($P = 0.45$)	
E_2	0.26 ($P = 0.02$)	−0.27 ($P = 0.02$)	−0.24 ($P = 0.03$)	−0.15 ($P = 0.19$)	0.34 ($P = 0.02$)

Spearman correlation coefficients. T_1, first visit; AMH, anti-müllerian hormone; AFC, antral follicle count; FSH, follicle-stimulating hormone; E_2, estradiol.

lower AMH levels, a lower AFC, higher FSH concentrations, and a lower inhibin B level at T_1. No significant difference in time interval between the two visits for women with a regular cycle and women with an irregular cycle [3.9 (3.1-4.7) y vs 3.9 (3.3-4.7) y, $P = 0.53$] was present. Also, there were no differences in body mass index (25.1 (20.4-31.3) kg/m^2 vs 24.5 (20.1-29.9) kg/m^2, $P = 0.37$) or in the percentage of smokers (22% vs 7%, $P = 0.28$) between the women with regular cycles and the women with irregular cycles.

The correlation coefficients of the ovarian reserve markers measured at T_1 are presented in Table 2. AMH and AFC were highly correlated with age and also highly correlated with each other. Lower but significant correlations were present when FSH was correlated with age, AMH, and AFC. Inhibin B was only significantly correlated with E_2.

In Table 3, the results of the univariable logistic regression analysis are presented. Age, AMH, AFC, FSH, and inhibin B were significantly associated with the occurrence of the menopausal transition within 4 years. Based on the ROC$_{AUC}$, AMH, AFC, and age had the best discriminative potential as a single predictor of the menopausal transition. Inhibin B and FSH had a somewhat lower but still discriminative ROC$_{AUC}$. E_2 levels were not significantly associated with the outcome measure. Neither body mass index [OR (95% CI) 0.93 (0.80-1.09; $P = 0.37$)] nor smoking [OR (95% CI) 0.27 (0.03-2.21; $P = 0.22$)] were significantly associated with the occurrence of the menopausal transition.

Because age is known and predictive for cycle irregularity, it is of interest whether extra testing improves prediction. Therefore, we analyzed whether each of the ovarian reserve tests contributed significantly to the prediction of the menopausal transition after correcting for age (Table 3). Both AMH and inhibin B were significantly associated with cycle irregularity, taking the effect of age into account. The ROC$_{AUC}$ changed from 0.82 for age as a single predictor to, respectively, 0.89 and 0.88 when AMH or inhibin B was added. If age was known, no significant effect of FSH or AFC was present.

In the multivariable stepwise logistic analysis, the variables AMH, inhibin B, and age were selected in that order. The ROC$_{AUC}$ increased from 0.87 for AMH alone to 0.92 for the total model. The bootstrap procedure indicated that AMH and inhibin B were selected in the majority of cases (respectively, 86% and 72%), whereas age was selected in approximately half of the samples (51%). The other variables AFC, FSH, and E_2 were selected in only a minority of cases (16%, 11%, and 9%, respectively).

DISCUSSION

Menopausal transition (cycle irregularity) is an important reproductive milestone because, at that stage, fertility is most severely compromised.[2] However, during the years before, the reproductive capacity is already impaired. The fact that the median age of (almost) end of fertility is 41 years, whereas the median age of the menopause transition is 46 years, confirms this notion.[2] Therefore, when a woman can be identified to develop an irregular cycle pattern within the three to five years ahead, she is likely to be in her late reproductive phase,[10] with a low chance of becoming pregnant.

Our results suggest that the novel marker AMH is a promising predictor for the occurrence of the menopausal transition in the subsequent 3 to 5 years. Serum AMH concentrations measured at T_1 differed significantly between women who continued to have regular cycles and those who developed an irregular cycle pattern. It was one of the best single predictors and, in more than 80% of the cases, AMH was selected when using the bootstrap procedure.

The other markers, except E_2 levels, also differed between these two groups of women. Inhibin B had a significant additional contribution to the prediction if AMH and age were taken into account, and inhibin B was selected in more than 70% of cases in the bootstrap procedure. AFC was also predictive for cycle irregularity. However, when correcting for AMH or age, AFC was no longer predictive due to the high correlation with both variables (Table 2). FSH performed less in

TABLE 3. *Predictive capacity of ovarian reserve markers assessed at T_1 for outcome cycle irregularity within 4 years*

	OR (95% CI)	P	ROC$_{AUC}$ (95% CI)
Univariate analysis			
Age (per y)	1.50 (1.16–1.94)	0.002	0.82 (0.71–0.93)
AMH (per 0.1 µg/L)	0.77 (0.65–0.92)	0.003	0.87 (0.79–0.96)
AFC (per follicle)	0.71 (0.56–0.90)	0.005	0.80 (0.69–0.91)
FSH (per IU/L)	1.20 (1.03–1.39)	0.016	0.72 (0.56–0.88)
Inhibin B (per ng/L)	0.98 (0.97–0.99)	0.003	0.76 (0.63–0.89)
E_2 (per pmol/L)	1.00 (0.998–1.006)	0.27	0.55 (0.35–0.75)
Multivariable analysis, after adjusting for age			
AMH (per 0.1 µg/L)	0.80 (0.67–0.96)	0.02	0.89 (0.81–0.97)
AFC (per follicle)	0.84 (0.62–1.14)	0.26	0.84 (0.73–0.94)
FSH (per IU/L)	1.14 (0.98–1.33)	0.09	0.84 (0.74–0.94)
Inhibin B (per ng/mL)	0.98 (0.97–0.99)	0.005	0.88 (0.78–0.98)
E_2 (per pmol/L)	1.00 (0.997–1.001)	0.64	0.83 (0.72–0.94)
Multivariable analysis, all variables			
AMH (per 0.1 µg/L)	0.81 (0.67–0.99)	0.04	
Inhibin B (per ng/L)	0.98 (0.97–0.997)	0.02	
Age (per y)	1.39 (0.97–1.98)	0.07	
			0.92 (0.86–0.99)[a]

[a]ROC$_{AUC}$ value for model.

predicting cycle irregularity in comparison with AMH, inhibin B, and age because it was selected in only a minority of cases in the bootstrap procedure. E_2 levels were not significantly related to the menopausal transition. The interassay coefficients of variation of the E_2 assay were poorest, which may contribute to this result. However, in daily practice, E_2 assays will always show this variation, and E_2 measurements will, therefore, continue to be not very useful.

The process of reproductive aging is related to the size of the ovarian follicle pool.[2,6,9] A marker giving a good reflection of the follicle pool is potentially suitable for the individual prediction of ovarian senescence. AMH is exclusively produced by granulosa cells of primary, secondary, and antral follicles and, therefore, is a good candidate.[17,18,23,24] In contrast, AFC, inhibin B, and FSH are presumed to reflect directly (AFC, inhibin B) or indirectly (FSH) the size of the antral follicle pool only. AMH was the only ovarian reserve test changing longitudinally in young women, and postmenopausal women all have undetectable AMH levels.[17] Furthermore, serum AMH concentrations are related to the number of oocytes retrieved after controlled ovarian hyperstimulation in IVF treatment,[18,25] which can be considered as a reflection of ovarian reserve.[26]

In the multivariable analysis, the measurement of inhibin B levels added predictive information after controlling for AMH (Table 3). It is assumed that low inhibin B levels are indicative for a decline in the number of recruited antral follicles.[15] Therefore, it seems surprising that inhibin B contributed independently to the prediction after AMH was taken into account because

AMH also gives a reflection of the antral follicle cohort. The low correlation of inhibin B with AMH (Table 2) and with AFC[27,28] reflects that inhibin B may not simply represent the number of antral follicles but is more related to changes in the functional state of the follicles with aging.

A marker that already demonstrates a considerable change during the years when the cycle pattern is still regular better identifies the women who are in their late reproductive stage. There are indications that FSH increases only substantially when the cycle pattern has become irregular.[14] Serum AMH concentrations already decline in younger women still having a regular cycle.[17]

Chronological age showed a good performance in predicting the menopausal transition; therefore, the additional value of measuring AMH and inhibin B may be questioned. However, women from this study population were of normal fertility in the past and were thought to represent the normal physiological decline of ovarian function.[27] Therefore, age may have performed better in this positively selected study population than it would have in a random population, which does contain women with infertility problems. On the other hand, even in the present study population, a biological variation in reproductive status within age categories will be present and, indeed, AMH and inhibin B independently added prognostic information. It is likely that, in a population also containing infertile women, AMH and inhibin B are even more important predictors.

The results of this study may contribute to the debate about the recently proposed staging system for female

reproductive aging.[10] Currently, FSH is incorporated in this staging system as an endocrine marker to discern the late reproductive phase. Elevated FSH levels and regular menstrual cycles are considered to characterize these years before the transition to menopause. However, FSH performed less well in predicting the menopause transition and was selected only in a minority of cases in the bootstrap procedure, in contrast with AMH and inhibin B. The authors of STRAW already indicated that there might be better markers than FSH to discern the late reproductive phase. FSH was chosen because it can be readily assayed in most laboratories, unlike AMH and inhibin B.[10]

Although this was a small study with a limited number of events, it seems that AMH is a better candidate than FSH for predicting the menopausal transition. However, due to the small size, no cutoff values and recommendations can be given at this stage, and larger studies should follow.

CONCLUSION

Serum AMH concentrations alone or in combination with inhibin B are predictive of the occurrence of the menopausal transition in subsequent years. Potentially they seem better markers to reflect the reproductive status of individual women than FSH.

REFERENCES

1. Brambilla DJ, McKinlay SM. A prospective study of factors affecting age at menopause. *J Clin Epidemiol* 1989;42:1031–1039.
2. te Velde ER, Pearson PL. The variability of female reproductive ageing. *Hum Reprod Update* 2002;8:141–154.
3. den Tonkelaar I, te Velde ER, Looman CW. Menstrual cycle length preceding menopause in relation to age at menopause. *Maturitas* 1998;29:115–123.
4. Eijkemans MJ. *Fertility in Populations and in Patients: Population Studies on Natural Fertility and Prediction of Treatment Outcome in Anovulatory Patients* [thesis]. Rotterdam, The Netherlands: Erasmus University; 2004.
5. Wood JW. Fecundity and natural fertility in humans. *Oxf Rev Reprod Biol* 1989;11:61–109.
6. Faddy MJ, Gosden RG, Gougeon A, Richardson SJ, Nelson JF. Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. *Hum Reprod* 1992;7:1342–1346.
7. van Noord-Zaadstra BM, Looman CWN, Alsbach H, Habbema JDF, te Velde ER, Karbaat J. Delaying childbearing: effect of age on fecundity and outcome of pregnancy. *BMJ* 1991;302:1361–1365.
8. van Kooij RJ, Looman CW, Habbema JD, Dorland M, te Velde ER. Age-dependent decrease in embryo implantation rate after in vitro fertilization. *Fertil Steril* 1996;66:769–775.
9. Richardson SJ, Senikas V, Nelson JF. Follicular depletion during the menopausal transition: evidence for accelerated loss and ultimate exhaustion. *J Clin Endocrinol Metab* 1987;65:1231–1237.
10. Soules MR, Sherman S, Parrott E, et al. Executive summary: Stages of Reproductive Aging Workshop (STRAW), Park City, Utah, July 2001. *Menopause* 2001;8:402–407.
11. Sherman BM, West JH, Korenman SG. The menopausal transition:

12. Perez MM, Gromoll J, Behre HM, Gassner C, Nieschlag E, Simoni M. Ovarian response to follicle-stimulating hormone (FSH) stimulation depends on the FSH receptor genotype. *J Clin Endocrinol Metab* 2000;85:3365–3369.
13. Schipper I, de Jong FH, Fauser BC. Lack of correlation between maximum early follicular phase serum follicle stimulating hormone concentrations and menstrual cycle characteristics in women under the age of 35 years. *Hum Reprod* 1998;13:1442–1448.
14. Burger HG, Dudley EC, Hopper JL, et al. Prospectively measured levels of serum follicle-stimulating hormone, estradiol, and the dimeric inhibins during the menopausal transition in a population-based cohort of women. *J Clin Endocrinol Metab* 1999;84:4025–4030.
15. Welt CK, McNicholl DJ, Taylor AE, Hall JE. Female reproductive aging is marked by decreased secretion of dimeric inhibin. *J Clin Endocrinol Metab* 1999;84:105–111.
16. Scheffer GJ, Broekmans FJ, Dorland M, Habbema JD, Looman CW, te Velde ER. Antral follicle counts by transvaginal ultrasonography are related to age in women with proven natural fertility. *Fertil Steril* 1999;72:845–851.
17. de Vet A, Laven JSE, de Jong FH, Themmen APN, Fauser BCJM. Anti-müllerian hormone serum levels: A putative marker for ovarian aging. *Fertil Steril* 2002;77:357–362.
18. Van Rooij IA, Broekmans FJ, te Velde ER, et al. Serum anti-müllerian hormone levels: a novel measure of ovarian reserve. *Hum Reprod* 2002;17:3065–3071.
19. Mitchell ES, Woods NF, Mariella A. Three stages of the menopausal transition from the Seattle Midlife Women's Health Study: toward a more precise definition. *Menopause* 2000;7:334–749.
20. Groome NP, Illingworth PJ, O'Brien M, et al. Measurement of dimeric inhibin B throughout the human menstrual cycle. *J Clin Endocrinol Metab* 1996;81:1401–1405.
21. Long WQ, Ranchin V, Pautier P, et al. Detection of minimal levels of serum anti-müllerian hormone during follow-up of patients with ovarian granulosa cell tumor by means of a highly sensitive enzyme-linked immunosorbent assay. *J Clin Endocrinol Metab* 2000; 85:540–544.
22. Harrell FE Jr, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat Med* 1996;15:361–387.
23. Durlinger AL, Visser JA, Themmen AP. Regulation of ovarian function: the role of anti-müllerian hormone. *Reproduction* 2002; 124:601–609.
24. Fanchin R, Schonauer LM, Righini C, Guibourdenche J, Frydman R, Taieb J. Serum anti-müllerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3. *Hum Reprod* 2003;18:323–327.
25. Seifer DB, MacLaughlin DT, Christian BP, Feng B, Shelden RM. Early follicular serum müllerian-inhibiting substance levels are associated with ovarian response during assisted reproductive technology cycles. *Fertil Steril* 2002;77:468–471.
26. Beckers NG, Macklon NS, Eijkemans MJ, Fauser BC. Women with regular menstrual cycles and a poor response to ovarian hyperstimulation for in vitro fertilization exhibit follicular phase characteristics suggestive of ovarian aging. *Fertil Steril* 2002;78:291–297.
27. Scheffer GJ, Broekmans FJ, Looman CW, et al. The number of antral follicles in normal women with proven fertility is the best reflection of reproductive age. *Hum Reprod* 2003;18:700–706.
28. Yong PY, Baird DT, Thong KJ, McNeilly AS, Anderson RA. Prospective analysis of the relationships between the ovarian follicle cohort and basal FSH concentration, the inhibin response to exogenous FSH and ovarian follicle number at different stages of the normal menstrual cycle and after pituitary down-regulation. *Hum Reprod* 2003;18:35–44.

analysis of LH, FSH, estradiol, and progesterone concentrations during menstrual cycles of older women. *J Clin Endocrinol Metab* 1976;42:629–636.