Serum antimüllerian hormone levels best reflect the reproductive decline with age in normal women with proven fertility: a longitudinal study

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Objective: The aim of this study was to assess which of the basal ovarian reserve markers provides the best reflection of the changes occurring in ovarian function over time (i.e., reproductive aging).
Design: Prospective longitudinal study.
Setting: Healthy volunteers in an academic research center.
Patient(s): Eighty-one women with normal reproductive performance during the course of their lives were longitudinally assessed. In this select group of women, becoming chronologically older was considered as a proxy variable for becoming older from a reproductive point of view.
Intervention(s): The women were assessed twice, with on average a 4-year interval (T_1 and T_2). The number of antral follicles on ultrasound (AFC) and blood levels of antimüllerian hormone (AMH), FSH, inhibin B, and E_2 were assessed.
Main Outcome Measure(s): Longitudinal changes of the markers mentioned and the consistency of these parameters over time.
Result(s): The mean ages at T_1 and T_2 were 39.6 and 43.6 years, respectively. Although AFC was strongly associated with age in a cross-sectional fashion, it did not change over time. The AMH, FSH, and inhibin B levels showed a significant change over time, in contrast to E_2 levels. The AMH and AFC were highly correlated with age both at T_1 and T_2, whereas FSH and inhibin B predominantly changed in women more than 40 years of age. To assess the consistency of these parameters over time, we investigated whether a woman's individual level above or below the mean of her age group at T_1 remained above or below the mean of her age group at T_2. Serum AMH concentrations showed the best consistency, with AFC as second best. The FSH and inhibin B showed only modest consistency, whereas E_2 showed no consistency at all.
Conclusion(s): These results indicate that serum AMH represents the best endocrine marker to assess the age-related decline of reproductive capacity. (Fertil Steril® 2005;83:979–87. ©2005 by American Society for Reproductive Medicine.)

Key Words: Reproductive aging, AMH, AFC, FSH, inhibin B

With increasing age there is a decline in a woman's reproductive function, which is assumed to be determined by the decline of the ovarian follicle pool and the quality of the oocytes within (ovarian reserve) (1). Menopause is reached at a median age of 51 years, when the follicle pool is (almost) depleted (2). Given the considerable individual variability in age at menopause (3), it is plausible that the process of follicle decline shows a comparable variability. Hence, for women of similar ages, large differences in their ovarian reserve are likely to exist.

Various endocrinological and sonographic markers have been used to assess the ovarian reserve. First, an increase in FSH levels in women over 35 years was observed (4). Subsequently, it was shown that inhibin B levels diminished with advancing age, presumably due to a reduction of the recruited cohort of the antral follicle pool during the follicular phase of the cycle (5–7). In addition, changes in E_2 levels have been described in women of advanced reproductive age. Both an increase (8) and a decline (9) were reported. The size of the antral follicle cohort can be directly assessed by ultrasound (10), and the observed pattern of its decline appears to correspond with that of the primordial

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follicle pool (11). Recently, serum antimüllerian hormone (AMH) levels have been introduced as a novel measure of ovarian reserve. AMH is a product of granulosa cells of the preantral and antral follicles (12, 13). Serum AMH levels decline with age and are related to the number of antral follicles and to the ovarian response after ovarian hyperstimulation (14–18).

Previously, we have shown in a cross-sectional study that the number of antral follicles assessed by ultrasonography (AFC) better reflects chronological age in normal women with regular cycles and proven normal fertility (19), compared to other ovarian reserve tests. Because these women were selected on the basis of normal reproductive performance during the course of their lives, it was assumed that their ovarian function reflects the gradual age-related decline of normal reproductive capacity. Therefore, their chronological age was considered to approximate their reproductive age. However, as indicated in such a population the variation of reproductive capacity in women of the same age category is likely to exist.

This previous study was the beginning of a continuing longitudinal study. Women were asked to return every 4 years for a new assessment of their ovarian reserve. With such a design, it is possible to study changes in ovarian reserve markers in a prospective manner in individual women. Because the same assumptions were made for the longitudinal part of this study, becoming 4 years older in chronological age is considered to approximate an increase of 4 years in reproductive age for these women with proven fertility. This present study was designed to assess the change in levels and values of ovarian reserve over time. The aim of the study was to determine which of the ovarian reserve tests presently available best reflects the process of reproductive aging in individual women.

MATERIALS AND METHODS

Subjects

In 1996 and 1997 female volunteers were recruited for a longitudinal cohort study through advertisement in local newspapers. The inclusion criteria have been described before (11, 19). In short, the women had to be 25–46 years of age and to have a regular menstrual cycle of 21–35 days. They had to have a proven natural fertility, which was defined as having established one or more pregnancies within 1 year after stopping contraception. This pregnancy resulted in a normal delivery at term. Moreover, they never had ovarian surgery or ovarian abnormalities and they had stopped hormonal contraception for 3 months or more before entering the study.

Of the 162 volunteers included in 1996–1997 (11, 19), 82 agreed to continue. The remaining 80 women did not participate in the longitudinal part of this study because they did not want to stop hormonal contraception (n = 10), were pregnant or breastfeeding (n = 3), had intercurrent disease (n = 3), had no time (n = 8), or had moved away from the area (n = 37). For 19 volunteers the reason for not participating was not known. The women not taking part in the follow-up study were on average 4 years younger than the women who did participate.

The Institutional Review Board approved of the study and written informed consent was obtained from all participants. The volunteers received monetary compensation for participating.

Study Design

The 82 women participating in this longitudinal study were tested at T_1 (1996/1997) and at T_2 (2001) during the early follicular phase of the menstrual cycle (cycle days 2, 3, or 4). If at T_2 no menstrual period had taken place during the past 3 months because menopausal transition had been reached, a visit was planned at the woman's convenience. During both visits a transvaginal ultrasound was performed using a 7.5-MHz probe on a Toshiba Capasee SSA-220A (Toshiba Medical Systems Europe BV, Zoetermeer, The Netherlands). At T_1 the ultrasound was performed by G.J.S. and at T_2 by I.A.J.R. The number of antral follicles was assessed and all follicles up to 10 mm were included in the analysis, as described previously (11).

During the same visit blood was drawn. Serum and plasma were separated and stored at −20°C for later estimation of levels of AMH, FSH, inhibin B, and E_2. The plasma and serum samples collected at T_1 and T_2 of each volunteer were assessed in the same assay run.

Some of the women reached menopausal transition, because cycle irregularity occurred after these women had normal regular cycles when they were included at baseline. There is no available uniform definition for menopausal transition, although recently some definitions based on increasing variability in cycle length have been proposed (20, 21). We defined menopausal transition in two ways. The mean cycle length is between 21 and 35 days, but in the last half year the next cycle is not predictable within 7 days. Or the mean cycle length is <21 or >35 days in the last half year.

None of the women in the study used hormonal replacement therapy (HT) for menopausal symptoms. Two women stopped hormonal contraception 3 months before inclusion. They regained regular cycles and continued to do so for 6 months.

Assays

Levels of FSH and E_2 were measured in plasma with the AxSYM immunoanalyzer (Abbott Laboratories, Abbott Park, IL). The World Health Organization Second International Reference Preparation for human FSH (78/549) was used as a standard for the FSH assay. For FSH, interassay coefficients of variation were found to be 5.7%, 5.7%, and