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Antral follicle counts are related to age at natural fertility loss and age at menopause

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Abstract

Objective: The variability in ultrasound-based antral follicle counts sized 2-10 mm after allowing for age-related decline is considerable. This may represent differences in actual reproductive age among women. This hypothesis was tested by cohort comparison for distribution of age at occurrence of reproductive events.

Design: A model with a nonlinear mean decline with age was fitted to antral follicle counts (AFC) obtained in 163 regularly cycling fertile volunteers. Ages at last child birth and menopause were *predicted* from the individual AFC by using thresholds to represent these events and the model for decline with age. Distributions of the *observed* ages at last childbirth (proxy variable for loss of natural fertility) and ages at menopause were obtained from the BALSAC demographic database and the Prospect-EPIC study, respectively. The observed distributions were compared with the predicted distributions by using visual comparison and quantile-quantile plots. Predictions of age at last child and age at menopause were done using percentiles of the modeled AFC distribution for given age, and corresponding percentiles of the predicted distributions of age at these reproductive events, with predictions following from the position of a woman's AFC relative to these percentiles.

Results: The *predicted* distributions of age at last child and age at menopause showed good agreement with the *observed* distributions in the BALSAC and EPIC cohort. Compared with age alone, antral follicle counts gave some additional information for individual prediction of age at last child and menopause.

Conclusions: The link between declining antral follicle counts and reproductively significant events like loss of natural fertility and menopause is strengthened by the high degree of similarity among the predicted and observed age distributions. Predictive usefulness of this relationship in a clinical setting may be more marginal, except in the case of women who have low AFCs for their age.

Key Words: Antral follicle count – BALSAC – Prospect-EPIC – Menopause – Ovarian aging – Volunteer.

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Reproductive aging considerably increases the risk of infertility in women. The age-related loss of female fecundity is almost completely caused by changes in the ovaries. The reproductive capacity of an individual woman is thought to be determined by two major components: the number of remaining follicles in the ovary and the quality of the oocytes held within these follicles. The stock of follicles declines with advancing age, a pro-

cess that has already started in fetal life.[1] This loss of follicles dictates reproductive events such as the occurrence of cycle irregularity and menopause. Menopause, for instance, coincides with the finding of only a few hundred follicles left in the ovaries.

The assessment of the quantity of follicles in the ovaries has been the subject of several studies. The number of follicles in the ovaries can be estimated by counting small antral follicles in the size range 2-10 mm, as visualized by transvaginal sonography. From the work by Gougeon it has become clear that numbers of antral follicles are related to numbers of primordial follicles within both ovaries.[2] Moreover, the number of small antral follicles decreases with age.[3,4] The pattern of decline in the number of antral follicles with age shows a great deal of similarity with the decline in total follicle numbers, as described in the studies by Faddy and Gosden.[4,5] Therefore, antral follicle count (AFC) in both ovaries assessed by transvaginal ultrasound may be an adequate marker of quantitative ovarian reserve and, thereby, of reproductive age.

The occurrence of menopause is thought to be largely dictated by changes in follicle numbers, as described earlier. Menopause is the only reproductive event after menarche that is clearly recognizable. The mean age at which this event occurs is 51 years. Among women, age at menopause shows a considerable degree of variability.[6] Other reproductive life events, such as the onset of cycle irregularity, which is typical of the climacteric woman, are quite strongly related to age at menopause.[7] Finally, the age at which a woman will give birth to her last child has been studied in several populations where contraceptive measures have not been applied because of culture or religion.[8] These studies show that women deliver their last child at a mean age of 41, but variability around this milestone is also considerable. Age at last child can be considered as representative of the age at which a woman has lost her natural fertility.

The question can be asked whether the occurrence of reproductive events like menopause, onset of cycle irregularity, or age at natural fertility loss is related to the number of remaining follicles in the ovaries. From studies on postmortem histologic counts of follicles, a predictive distribution of the ages at which menopause occurs corresponded to total follicle numbers declining below a threshold number of 1,100 follicles and showed striking similarity with the distribution of age at menopause, as observed in the studies by Treloar.[5]

To investigate whether ultrasound-based antral follicle number is an indicator of reproductive age, prediction of menopause from AFCs could be studied and compared with observed age at menopause, in a similar way to that from histologic counts. Moreover, if the distribution of age at last child corresponds to antral follicles declining below a threshold number, then this number of antral follicles could serve as an indicator for the age at natural fertility loss. Hence, antral follicle numbers could be used to predict the distribution of age at natural fertility loss and could be compared with observed distributions in natural populations.

The aim of the present study was first to obtain support for the hypotheses formulated above by comparing *predicted* ages at which natural fertility loss and menopause occur, from AFCs obtained in normal fertile women, with the *observed* ages at last child, from data on a natural Canadian population, and *observed* ages at menopause, from a study of Dutch women. Second, the usefulness of AFCs as predictors of future reproductive events such as loss of natural fertility and menopause was investigated.

METHODS

Study design

To answer the questions as presented, three sources of information were used.

First, a group of 163 healthy, predominantly Caucasian, regularly cycling, fertile women volunteers was recruited. Inclusion criteria were published earlier.[4] Briefly, healthy women aged 25-46 years were recruited through advertisements in local newspapers. Volunteers were enrolled in the study protocol if they met all of the following criteria: (1) regular menstrual cycles, with mean length varying from 21 to 35 days; (2) biphasic basal body temperature; (3) proven natural fertility by having carried at least one pregnancy to term; (4) each of the pregnancies established within 1 year after the interruption of contraceptive methods; (5) no evidence of endocrinologic disease; (6) no history of ovarian surgery; (7) no ovarian abnormalities, as assessed by vaginal ultrasound; and (8) cessation of hormonal contraception 2 months before entering the study protocol. All volunteers were subjected to transvaginal sonography of the ovaries performed by the same observer (GS) on either cycle day 2, 3, or 4, using the 7.5 MHz transvaginal probe on a Toshiba Capasee SSA-220A (Toshiba Medical Systems Europe BV, Zoetermeer, the Netherlands).[4] Examination of the ovary was done by scanning from the outer to the inner margin. All round or oval sonolucent structures confined within the contour of the ovary were regarded as follicles and measured and counted as such. For the data analysis, only follicles of 2-10 mm were included.

Intra- and interobserver agreement for follicle counts is adequate as shown by intraclass correlation coefficients of 0.99 and 0.98, respectively.[9] The Institutional Review Board approved the study, and written informed consent was obtained from all participants. The volunteers received monetary compensation for participating.

Second, to study the relationship between age and loss of natural fertility, data were used from women born between 1850 and 1899 in the Saguenay-Lac-St-Jean region of Quebec, Canada, who married and died in the region. These data, from church registers, on birth, baptism, marriage, and death, are contained in the BALSAC demographic database (BALSAC project at the University of Quebec in Chicoutimi).[10,11] The birth cohort of 1850 to 1899 (n = 1,040) was chosen to ensure that all women would have finished their reproductive life and to exclude any effect of the use of contraceptive measures. The majority of women in the BALSAC database had one or more sisters who were also contained in the database. To avoid statistical dependence between participants, we selected only one of the female children from a family, according to a random procedure.[12] The data were further reduced by including only women whose marriage lasted at least until the age of 53 (the last age at which a woman in this sample gave birth to a child), to eliminate a possible limitation of fertility potential due to early ending of a marriage. The age at last child among women in this database was used as a proxy-variable for age at which natural fertility is lost.

Third, a sample of 3,483 Dutch women participating in the Prospect-EPIC (European Prospective Investigation into Cancer and Nutrition) study was used to estimate a distribution of the age at menopause.[13-15] The Prospect-EPIC cohort recruited women 50 to 70 years of age from an ongoing nationwide breast cancer screening program conducted in the Netherlands. Data on reproductive history were obtained from a general questionnaire in which demographic and medical information was sought. Menopause was defined as a condition in which a woman has not experienced a spontaneous menstrual bleeding for more than 12 months. Only women who had given birth to at least one child and in whom occurrence of menopause was not obscured by hormonal medication were selected for the present sample of postmenopausal women to create a high degree of comparability with the two other cohorts of women used in this study. To prevent underrepresentation of women who reach menopause late in their life, only women 58 years and older were selected for the sample. Finally, women who had undergone hysterectomy or ovarian surgery were excluded. From a total cohort size of 17,357,[14] 3,483 women who met all the criteria described were selected and used in the analysis.

Analysis

The AFCs obtained from the study of normal fertile volunteers were plotted against age. A model that showed an initial linear decline in mean follicle counts followed by an exponential decline with asymptote at zero was used to describe the data, assuming a negative binomial residual distribution. It was considered that this model would represent the change in follicle numbers with age for individuals in the population and that the variation in AFCs would correspond to variation in the future occurrence of reproductive events. Women with low follicle numbers would reach menopause earlier than those with high numbers and the same age.

This model was then used to obtain predictive distributions of age at last child and age at menopause, assuming that these events were triggered by the AFC falling below certain thresholds. These distributions were then fitted to the BALSAC and EPIC data sets on age at last child and age at menopause, respectively. All model parameters were estimated by maximal likelihood. The predictive distributions so obtained were visually compared with the distributions of observed age at last child and age at menopause, and the agreement illustrated by QQ (quantile-quantile) plots, in which quantiles of the observed distribution were plotted against corresponding quantiles of the predicted distribution.

Predictions of age at last child and age at menopause for an individual woman were performed using quantiles of the fitted distributions. From a woman's AFC and age, she was placed in a percentile band (lower 5%, 5%-10%, 10%-25%, 25%-50%, or upper 50%) from the fitted model for age-related decline of AFCs, using the negative binomial residual distribution (for example, a woman aged 33 years would have 6 or fewer follicles with probability 0.1, and 9 or fewer with probability 0.25, etc). From the corresponding quintiles of the fitted distributions of age at last child and age at menopause, she could then be classified into one of five categories for age at last childbirth and menopause. In this way, age-based expectations for age at last childbirth and menopause could be adjusted on the basis of the observed antral follicle number.

RESULTS

A model with a linear mean decline in AFCs and a negative binomial residual distribution seemed to pro-

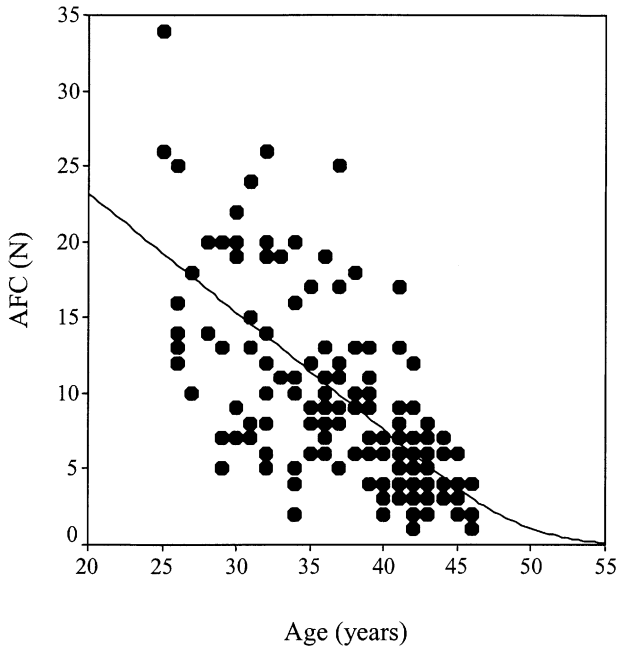

FIG. 1. Antral follicle counts as obtained in the cohort of fertile volunteers (dots) and fitted mean (line) for decline with chronological age, based on an initial linear decline, followed by an exponential approach to zero ($R^2 = 0.49$, indicating substantial residual variation).

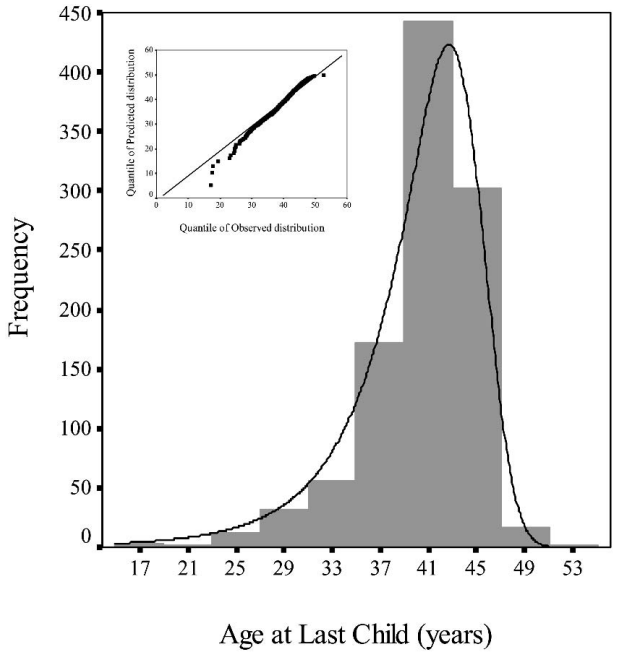

FIG. 2. Observed distribution of age at last child in a natural, religious population not applying contraceptive measures (BALSAC) (bars, n = 1,040) and predicted distribution from antral follicle counts in normal fertile women based on the modeled decline in Fig. 1 (curve). In the upper left corner is a quantile-quantile plot showing the quantiles of the distribution of observed ages at last child from women in the BALSAC cohort plotted against the corresponding quantiles of the distribution of predicted ages at natural infertility based on declining antral follicle counts in normal fertile women and a threshold of 6 follicles.

vide a good fit to the data,[16] with a deviance or goodness-of-fit statistic of 160.6 on 161 degrees of freedom. However, such a linear model would lead to there being no antral follicles left at the age of approximately 49 years. Therefore, an adjustment to the linear mean decline was made so that the mean AFC approached zero exponentially, as shown in Fig. 1. As such, this model describes the pattern of decline in antral follicle number: a woman with a low count at a young age will likely have exhausted her follicle pool at a relatively young age, whereas a woman with a high number when young will continue to have visible follicles until well into her 50s.

The mean age at last child, as calculated from the BALSAC data, was 40.6 years (SD = 4.4; median = 41.6 y). The distribution of ages at last child in this population (Fig. 2) exhibited clear skewing to the left, whereby more women tended to have their last child at a relatively young age compared with those who gave birth to their last child later in life. From the combined use of the AFC and BALSAC data, a threshold number of follicles for age at last child was estimated to be 6, whereby loss of natural fertility occurs when the AFC declines below this number. Comparison between the predictive distribution of age at last child based on follicle number decline and this threshold, and the observed distribution in the BALSAC population, is shown in the Q-Q plot in Fig. 2. This indicates good conformity between the distributions, except perhaps at very low ages (lower 2% or so of the distribution, or age less than 28 y).

In the EPIC data set, the mean age at which women experience menopause was 50.3 years (SD = 4.1; median = 51.0 y). The distribution of age at menopause also showed some skewing to the left, indicating that some women experienced ovarian arrest very early in life, perhaps due to factors such as chromosomal abnormalities (Fig. 3). From the AFC and EPIC cohort data, threshold numbers of follicles for menopause were estimated to be 0 or 1 (mean 0.17); ie, in 83% of women, menopause is triggered by the AFC reaching 0, and in 17% by AFC reaching 1. The resulting distribution of menopause ages predicted from the model for declining antral follicle numbers and these threshold numbers showed good agreement with the EPIC data distribution, as shown in the QQ plot in Fig. 3. This indicates that the observed age distribution is well matched by the predictive distribution.

In Fig. 4, the estimated percentile categories of AFC and age are depicted in conjunction with the corre-

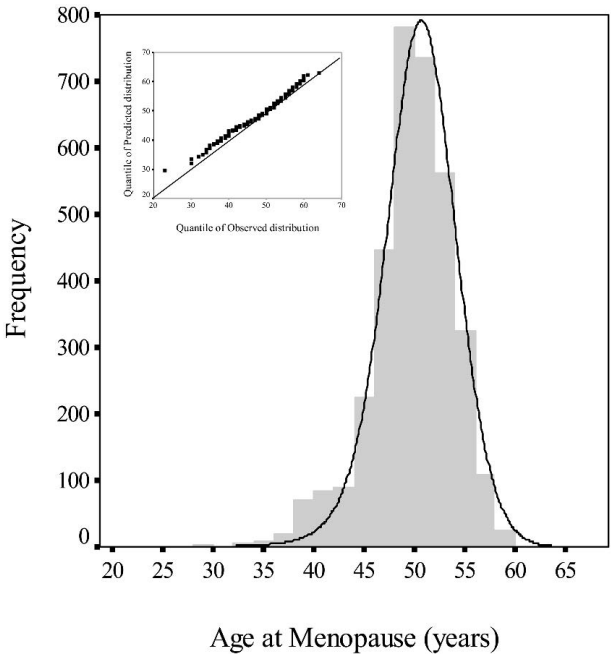

FIG. 3. Observed distribution of menopause ages in a cohort of women in a breast cancer screening program (EPIC), from whom reproductive data were obtained (bars, n = 3,483) and predicted distribution from antral follicle counts in normal fertile women based on the modeled decline in Fig. 1 (curve). In the upper left corner is a quantile-quantile plot, showing the quantiles of the distribution of observed ages at menopause from women in the EPIC cohort plotted against the quantiles of the distribution of predicted ages at menopause based on antral follicle counts in normal fertile women and a threshold of 0 or 1 follicle.

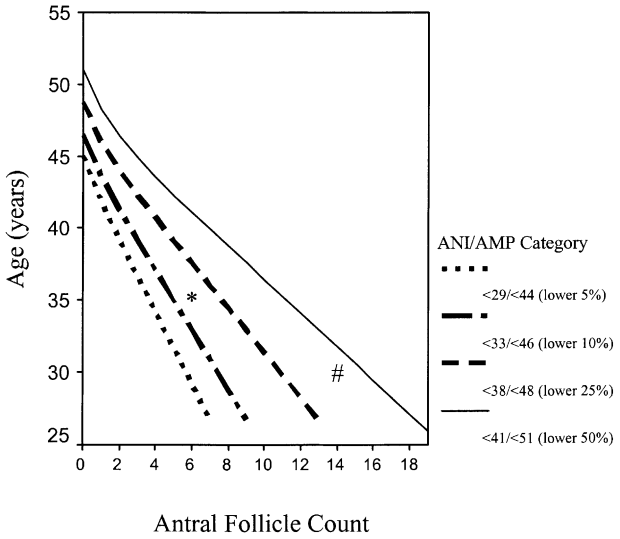

FIG. 4. Percentile categories of age at natural infertility and menopause predicted from age and antral follicle count (AFC) of women. *, a woman aged 35 with an AFC of 6 would be predicted to experience loss of natural fertility between the ages of 33 and 38, and menopause between the ages of 46 and 48; #, a woman 30 years old with an AFC of 15 could expect to experience natural fertility loss and menopause close to the median ages of 41 and 51 years. ANI, age at natural infertility, assumed equal to age at last child; AMP, age at menopause.

sponding age categories at which natural fertility loss and menopause are expected to take place. These show that women with an AFC low for their age are very likely to experience loss of natural fertility and menopause at a younger age than expected from age alone. At the same time, those with high follicle numbers for their age may expect to lose fertility and become postmenopausal at a much higher age than expected.

DISCUSSION

The observed number of antral follicles in the normal fertile group of volunteers showed a great deal of variation after allowing for age-related decline. This variation may be due to imprecision of the method used to count these follicles by transvaginal sonography. However, from intra- and interobserver studies, it seems that the agreement among measurements is quite good,[17] so that only a small amount of the observed variation could be accounted for by this imprecision. Therefore, most of the variation may be due to inter-individual differences in reproductive status of the women. A good level of agreement between the distributions of observed age at natural fertility loss and age at menopause on the one hand and the predicted distributions based on the follicle counts on the other was found in this study. These findings give support for the notion that, at the population level, antral follicle numbers do reflect reproductive status. Definitive evidence for this hypothesis can only be obtained from longitudinal studies in which the occurrence of menopause is prospectively assessed and women are subjected to regular measurements of their antral follicle numbers over a period of one or two decades. To date, such a study has not been done and would be a major undertaking.

The modeling of the follicle count data assumes that variation in AFC around the mean decline curve reflects differences in reproductive status and that threshold numbers of these follicles correspond to the occurrence of natural fertility loss and menopause. As for assessment of a threshold number of follicles for natural fertility loss, it is not unreasonable to say that counting antral follicles in natural populations where loss of natural infertility can be reliably identified would be virtually impossible. Therefore, a mathematical modeling approach to threshold estimation seems the only practical methodology here. As for menopause, the data from which the model parameters were estimated lacked information on numbers of follicles in the ovaries of climacteric and postmenopausal women. The modeling of low follicle counts and the estimated threshold for menopause both seem plausible when

data on postmenopausal women are considered. In the vast majority of such women, none or just a single antral follicle can be visualized by transvaginal sonography.[18,19] Whether this absence of follicles may have been the case for some years before the actual occurrence of menopause is a question that remains to be answered, although transversal studies suggest that this is not the case.[19]

The similarity between the predicted and observed distributions of age at loss of natural fertility and age at menopause are certainly noteworthy and give some support to a biologic link between the clear decline in follicle numbers with age and the reproductively significant events of menopause and last childbirth. However, at very young ages (less than 28 y), the correspondence between the predicted and observed distributions of age at last child is not so good (Fig. 2). This may be due to a lack of very young women in the study on normal fertile volunteers (minimal age 25 y), resulting in little information on follicle numbers in such women. Thus, low follicle numbers in these women might not be indicative of early occurrence of natural infertility. This is further exemplified in Fig. 4, where ages of the women were truncated at 25.

The variation in follicle counts among women could be explained at least in part by variation in the size of the antral follicle cohort from cycle to cycle. In a study by Scheffer on repeat measurements in the early follicular phase of subsequent cycles, it was shown that there was some variation among counts.[4] This suggests that any use of follicle counts in the counseling of women on their current or future reproductive potential may be marginal. However, if a woman had considerably fewer follicles than expected for her age, then predictions based on her AFC would be informative and, therefore, of potential value. The data presented in Fig. 4 suggest that AFCs indeed can be used as a correction to simple expectations based on a woman's chronological age. The use of ultrasound-based AFCs in the management of infertile couples has been the subject of a limited number of studies so far. Prediction of treatment-dependent pregnancy has been hampered by the fact that the occurrence of pregnancy is influenced by many largely unknown or uncontrolled factors. Our study affirms that there may be only a modest basis for pregnancy prediction here and that, like other ovarian reserve tests, outcome prediction will probably remain uncertain.[20-24] In contrast, the relation between AFCs and ovarian response to gonadotropin treatment in in vitro fertilization (IVF) has been shown to be quite adequate. Therefore, AFCs in combination with endocrine variables may well be of value as a predictive test for ovarian response in IVF, on the basis of which treatment schedules may be adapted.[25]

The question may be raised as to whether the historical database on age at last child (the BALSAC cohort) and the sample on menopause age (from the EPIC cohort) can be considered reliable and comparable sources. The BALSAC cohort stems from a Western Europe Caucasian population, and, as such, genetic determinants may not be different from those in the antral follicle cohort of women. As a peasant society, it was capable of fulfilling the basic needs for maintenance of life so that nutritional influences on cycle fecundity would be unlikely to have biased the observations on last childbirth.[26,27] From the existing literature on this closed community, it is clear that the position of a woman was one of domination by a male spouse. Women were excluded from politics and decision making, and their role was limited to that of a silent servant without power. The Catholic church disapproved of women working outside the home, and it was common practice to instruct the bride on the morning of her marriage not to refuse her husband and not to prevent births. The vast majority of women married in their early 20s, and a woman typically accepted a life of "owing herself" to her husband. Sexuality, therefore, was equated with procreation and motherhood. This is exemplified by the remarkably high fertility rates and also implies that procreation was only interrupted by lactational effects and the limits of natural fertility. Polygamy certainly would have been extremely rare in this community, implying that the natural fertility of all those women who remained married until the age of 53 was continuously challenged.[11,28] Altogether, the BALSAC cohort represents the best possible population for the study of normal female fertility decline.

Age at menopause depends on genetic and, to a much lesser extent, environmental factors, although conflicting reports exist regarding the latter.[29-33] This implies that no consistent set of factors regarding lifestyle or nutrition has been identified so far, apart from smoking. The selection from the EPIC cohort consists of women who stem from the same female Caucasian society as those in the normal fertile volunteer cohort in which AFCs were performed, albeit that mean date of birth is some 30 years apart. Neither genetic[30,34] nor environmental factors, therefore, would have been of great influence, although secular trends in the occurrence of menopause have been described.[33,35] From published literature,[4,14,36] it can be concluded that smoking behavior in the two cohorts is very much comparable (approximately 45% of both groups never smoked). Therefore, obvious bias regarding both the representa-

tiveness of the menopause age distribution in the EPIC sample, as well as the comparability of the two distributions, seems to be absent.

Socioeconomic changes in Western societies and consequent postponement of childbearing put pressure on reproductive physiologists to come up with tests that can truly assess a woman's reproductive status. Only through such tests can effective measures be taken to ameliorate age-related infertility. Currently, the development of such tests may be expected from large-scale screening for genes that determine the occurrence of reproductive events, such as menopause and cycle irregularity.[29] From these, the scale of an individual's reproductive life may become predictable. Poor ovarian response to adequate gonadotropin stimulation in IVF treatment has been repeatedly associated with early occurrence of cycle irregularity and menopause.[37,38] As poor response can be predicted by the use of AFCs, the use of the AFC in prediction of future reproductive events may prove possible. Combining age of the woman and follicle counts in a clinical setting will help to counsel women before undergoing in vitro fertilization, to adapt the treatment schedule, or to decide to refrain from such treatments if prospects become too unfavorable. Moreover, the use of AFC in combination with other ovarian reserve tests, such as basal FSH and inhibin B, will enlarge the predictive abilities.[25]

CONCLUSION

In summary, this study has shown that, at a population level, AFCs are clearly related to the occurrence of reproductive events such as menopause and age at last child. Therefore, antral follicle numbers may be considered as a reflection of reproductive status. If AFCs are low for age, then clinically useful assessment of reproductive capacity is suggested by the relationship between these variables.

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