Age-specific nomogram for the decline in antral follicle count throughout the reproductive period

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Objective: To investigate the relationship between antral follicle count (AFC) and chronological age and to establish normal values for AFC in women with regular menstrual cycles.
Design: Cross-sectional study.
Setting: University hospital.
Patient(s): Four hundred fifteen premenopausal women were recruited for the study. Data from 362 patients were available for the statistical analysis.
Intervention(s): AFC was measured by transvaginal ultrasound examination.
Main Outcome Measure(s): Estimating the relationship between AFC and age and developing the AFC nomogram.
Result(s): The analysis showed a linear decline in AFC with age; for every year increase in age, the median AFC decreases by 0.4. The AFC corresponding to the 5th, 25th, 50th, 75th, and 95th centiles for each age have been calculated.
Conclusion(s): A linear relationship of AFC to age was found. For the first time, a nomogram reporting normal and interquartile values for AFC, age by age, throughout the reproductive period has been provided. Until now, the interpretation of the measurement was mainly based on the individual experience of the operator, because no normative data were present. Therefore, the establishment of a nomogram of AFC values is the first step to counsel patients on a scientific basis. (Fertil Steril® 2011;95:684–8. ©2011 by American Society for Reproductive Medicine.)

Key Words: AFC, ovarian reserve, healthy women, normal values, reproductive period

The age-related decline in female reproductive function owing to the reduction of the ovarian follicle pool and the quality of oocytes has been well established. A reliable marker for the age at which subfertility will occur would have great potential value as a predictor of future reproductive life span. Because a considerable proportion of female subfertility arises because of postponed childbearing, any reliable measurement of ovarian reserve may also be of interest to women in general. The ideal marker would show a significant change in levels from adolescence to the late reproductive period and should enable age-independent prediction of an individual's reproductive life span and spontaneous pregnancy in the general population. Ovarian reserve tests include a variety of biochemical and ultrasound parameters (1–4). Currently available ultrasound tests of ovarian reserve include antral follicular count (AFC), ovarian volume, and Doppler assessment of ovarian blood flow (5–9).

AFC is the most commonly used ultrasound marker of ovarian reserve because of its ease of measurement and reliability (10, 11). The AFC consists of counting all small follicles in the range of 2–10 mm as visualised by transvaginal ultrasound examination.

Several studies of autopsy and surgical specimens showed that the number of antral follicles is related to the number of primordial follicles within the ovaries. In particular the number of small antral follicles decreases with age similarly to the number of primordial follicles (12, 13). Indeed, several studies have demonstrated that AFC declines with chronologic age in women (14–17), although it is still not clear whether this decline has a biphasic (15) or a linear pattern (7, 14, 18–20). Therefore, the sonographic study of AFC could offer valuable information on the status of ovarian reserve, because it reflects the continuous decline of the follicle pool with age.

The occurrence of menopause is thought to be dictated mainly by the change that occurs in follicle number. Based on studies from histological counts of follicles, a predictive distribution of the ages at which menopause occurs corresponds to a decrease of total follicle number declining below a threshold number ranging from 758–1,100 follicles (21, 22). Considering the strong positive correlation existing between the AFC and the extent of the follicle pool, AFC has been shown as a simple method of predicting the occurrence of menopause and thus the duration of the reproductive lifespan (16).

In clinical practice, AFC is particularly used in IVF clinics, where this marker may be informative on the ovarian response to gonadotropin administration. A low or high AFC has been invariably associated to increased risk for poor or hyperovarian response to gonadotropin, respectively (9, 23–25). Therefore, considering the wide use of AFC measurement in the daily clinical practice and the large number of conditions in which it can be used, it is essential to establish the normal values in the healthy female population. The

aim of the study was to investigate the relationship between AFC and chronologic age and to establish normal values for AFC in women with regular menstrual cycles.

MATERIALS AND METHODS

The goal of this cross-sectional study was to evaluate normal AFCs in normal menstruating women. The study was performed at the Institute of Obstetrics and Gynecology, University of Modena, between January 2008 and December 2009. Volunteers were prospectively recruited among patients attending the Institute of Obstetrics and Gynecology for the following reasons: participation in the national program of cervical cancer screening (n = 165), preconception counseling (n = 24), contraception counseling (n = 198), and request of tubal sterilization (n = 28).

Women were recruited when the following criteria were met: regular menstrual cycle (length, 25–35 days) with <5 days difference between cycles, age <50 years, and presence of both ovaries. Exclusion criteria were: history of hormone administration in the previous 6 months; history of pelvic inflammatory disease or ovarian surgery; history of infertility; known chronic, systemic, metabolic, and endocrine disease including hyperandrogenism. Patients with poor ultrasound visualization of ovaries, because of retrouterine or abnormal position and the presence at least one of cyst ≥20 mm, were excluded retrospectively. Informed consent from all the women and the institutional review board were obtained.

After recruitment, all patients attended our department in the follicular phase of the menstrual cycle. All ultrasound examinations were performed by one of three examiners (A.L.M., A.T., or S.G.) using the 6.5-MHz vaginal probe on an Esaote AU4 Idea (Esaote, Milan, Italy). Examination of the ovary was established by scanning from the outer margin to the inner margin. All follicles 2–10 mm in size were counted in each ovary. Follicular size is measured using the internal diameters of the area. The mean of two perpendicular measurements was assumed to be the follicular size. The sum of both counts produced the AFC.

Statistical Analysis

The AFC was plotted against the chronologic age, and the best fit line through the data was calculated. The effect of age on AFC was examined by both linear and nonlinear regression analysis. Models to describe AFC decay were constructed and evaluated with GraphPad Prism 5 (GraphPad Software, La Jolla, CA). For each type of model, we calculated values for the parameters that maximize the R^2 for that model. The models supplied by GraphPad Prism are those that are commonly reported in the scientific literature as models of datasets that rise and fall, such as pharmacodynamics, cell populations, and electromagnetic signals. The goodness-of-fit of the different models was determined by calculating the R^2 and the sum of squares error. When appropriate and possible, models were compared using the F-test and the Akaike information criterion.

To estimate the 5th, 25th, 75th, and 95th centiles, the CG-LMS method (26) was used. It is a model that expresses the centiles in terms of age-specific curves called L, M, and S. The M and S curves correspond to the median and coefficient of variation of AFC at each age, whereas the L curve allows for the age-dependent skewness of the distribution of the same trait. The value (y) of the AFC at a given age can be transformed into a standard deviation score (SDS): $SDS = [(y/M)^L – 1] ÷ (L \times S)$. The value of a centile can be computed from the L, M, and S values. For example, for the 10th centile (SDS = –1.28) of the AFC of a 19-year-old woman, we have $L = 0.425$, $M = 15.4$, and $S = 0.490$; therefore y (10th) = $15.4 \times (1 –1.28 \times 0.425 \times 0.490)^{1/0.425} = 7.4$. Centiles were calculated using the software LMS program version 1.29 (Medical Research Council, UK).

RESULTS

A total of 415 patients were recruited for the study, and 380 attended the clinic for ultrasound examination. After ultrasound examination, 18 were excluded because of poor visualization of the ovaries or the presence of ovarian cysts. Data were obtained from 362 patients for the statistical analysis.

The median age (25th–75th centile) of women was 38 (32–42) and median (25th–75th centile) of the AFC was 8 (5–12). The median, interquartiles, and lowest and highest values of ovarian AFCs for different age groups are illustrated in Figure 1.

AFC was correlated with age, and regression analysis revealed that age-related changes were best fitted by a linear function ($y = 25.87 – 0.46x$; F ratio, 211.7; $P < 0.001$; Fig. 2). The relationship between age and AFC was not significantly improved by nonlinear regression analysis. Age alone accounted for 37% of the variation in AFC.

Figure 3 shows AFC values as a function of age and the estimates of selected centiles (5th, 25th, 50th, 75th, 95th) based on a CG-LMS model that implies a linear relationship of AFC to age, and constant values for parameters S (0.490) and L (0.425). In particular, the value of L denotes that the distribution of AFC is positively skewed.

The CG-LMS model confirms a linear decline in AFC with age, where the median AFC decreases by 0.4 for every year increase in age. The median rate decrease for the number of follicles was of 2.4% per year. At the average age of menopause (51 years), the CG-LMS model predicts a median AFC of approximately 2 (5th–95th centile: 1–4). Based on these results, the AFCs corresponding to the 5th, 25th, 50th, 75th, 95th centiles can be calculated for each age and are shown in Table 1.

DISCUSSION

This study investigated the relationship between AFC and age on the largest general population published to date (n = 362). The majority of studies have investigated AFC in infertile patients (19, 27, 28) or patients with proven fertility (15, 16, 18, 29). Only two studies have been performed on the general population; however, one was limited to a small number of subjects (n = 31) (14). The second has been recently published and was performed on 252 patients ranging in age between 25 and 45 years (20).

FIGURE 1

Women were categorized in the following groups based on age: ≤30 years (n = 68), 31–35 years (n = 76), 36–40 years (n = 96), 41–45 years (n = 92), and 46–50 years (n = 30). The *horizontal line* represents the median, the *box* represents the 25th and 75th centiles, and the *vertical line* represents the lowest and highest values. ANOVA indicated a statistically significant difference in AFC values between the groups (F ratio, 41,907; $P < 0.001$). a = $P < 0.05$ vs. age group ≤30 years; b = $P < 0.05$ vs. age groups ≤30, 31–35, and 36–40 years.
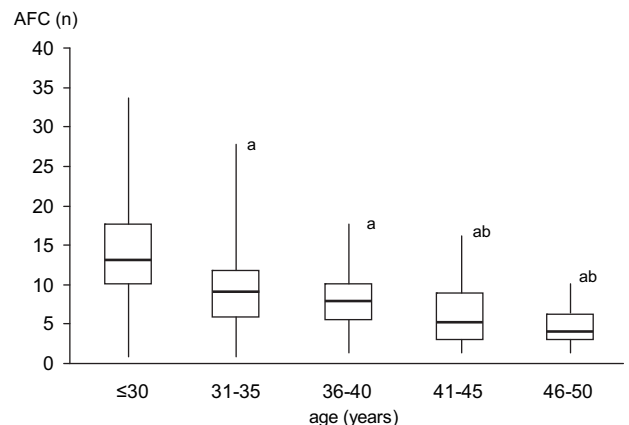
La Marca. Nomogram for the AFC. Fertil Steril 2011.