Age and the ovarian follicle pool assessed with transvaginal ultrasonography

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OBJECTIVE: We tested whether transvaginal ultrasonography could detect the age-related decrease in follicle counts that has been observed in autopsy studies.

STUDY DESIGN: Thirty-one healthy volunteers in three age groups (22 to 25, 30 to 33, and 39 to 42 years) underwent ultrasonography in the follicular and luteal phases of the menstrual cycle. At the conclusion of the study the 124 ovarian scans were randomly ordered and antral follicles ≥2 mm were counted by an evaluator unaware of age. Ordinary least-squares linear regression was used to estimate the associations of age with the total antral follicle count and with ln (1 + follicle count).

RESULTS: The numbers of antral follicles ≥2 mm decreased by about 60% between 22 and 42 years. Age-related decreases were similar for both phases of the cycle and held for both smaller (2 to 3.5 mm) and larger (>3.5 mm) follicles.

CONCLUSION: We hypothesize that ultrasonographically derived counts of follicles provide a measure of reproductive age that may help to predict age-related phenomena. (Am J Obstet Gynecol 1996;174:624-7.)

Key words: Transvaginal ultrasonography, ovarian follicles, age-related change

Recent improvements in transvaginal ultrasonography offer, for the first time, the opportunity to study the natural history of changes in the ovarian follicle pool in vivo. Until now, observations have been based on autopsy[1, 2] and surgical[3] specimens. These establish that the number of ovarian follicles decreases from fetal life to the menopause. In his classic autopsy study Block[1] observed that during the reproductive years the median number of follicles declined from about 104,000 in the early 20s to 33,000 in the early 30s to 7900 in the early 40s. A decline was also seen for antral (fluid-containing) follicles that were >1 mm in diameter; these are the small fraction (<0.5%) of the total pool that have developed and enlarged. The median number of these larger follicles decreased from 57 to 46 to 0 in the three age groups.

It is now possible to see developing follicles that have reached ≥2 mm in diameter by means of high-frequency transvaginal transducers.[4] We tested whether transvaginal ultrasonography could detect, during the reproductive years, the age-related decrease in antral follicles established in autopsy studies.

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Material and methods

Between October 1992 and March 1994, 31 healthy volunteers in three age groups, (22 to 25, 30 to 33, and 39 to 42 years), underwent transvaginal ultrasonography on days 4 to 6 of the follicular phase of the menstrual cycle and on days 19 to 25 of the luteal phase of the same cycle. All volunteers were white; all had regular menstrual cycles of 27 to 33 days with no missed menses, pregnancies, or hormonal medications in the past year; all were nonsmokers; none had a history of infertility or of polycystic ovary syndrome.

According to a study protocol that was approved by the Columbia-Presbyterian Medical Center Institutional Review Board, one of the authors (R.S.) performed and videotaped the scans using a C 9-5 ICT transducer of the ATL Ultramark 9 HDI (Advanced Technology Laboratories, Bothell, Wash.). The sonographer scanned through each ovary at a subjectively constant velocity, each one taking 5 to 20 seconds. To evaluate counts we mimicked histologic sectioning by printing evenly spaced frames that approximated 1 mm intervals. This procedure yielded 19 to 33 prints per ovary. At the conclusion of the study the 124 ovarian scans (31 subjects × 2 ovaries × 2 scans) were randomly ordered for evaluation by a second author (M.L.R.) who was unaware of the age of the patient or the stage of the menstrual cycle. Each follicle (i.e., sonolucent sphere ≥2 mm in diameter) was followed through several frames to determine its maximum diameter. For each phase separately the numbers of follicles in the left and right ovaries were summed to compute the total number of antral follicles for each subject.

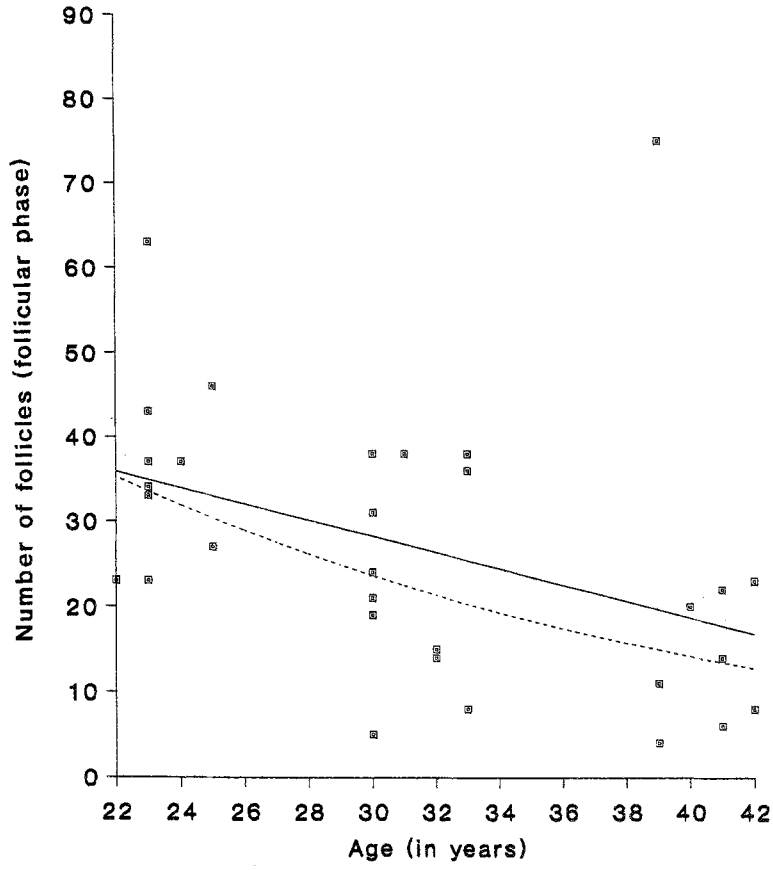

Fig. 1. Total number of follicles in follicular phase by age: observed and estimated values based on linear and logarithmic models. Linear model (——): Number of follicles = 28.3 − 0.952(Age 30), where 28.3 is estimated number of follicles at age 30. Logarithmic model (- - - -): Number of follicles = $e^{(3.20 - 0.048(\text{Age } 30))} - 1$, where $e^{3.20} - 1$ is estimated number of follicles at age 30.

Table I. Numbers of ovarian follicles (both ovaries) counted with transvaginal ultrasonography for three age groups and by phase of menstrual cycle

	Follicular phase (days 4-6)			*Luteal phase (days 19-25)*		
	Size of follicle			*Size of follicle*		
Age group	*2-3.5 mm*	*>3.5 mm*	*Total*	*2-3.5 mm*	*>3.5 mm*	*Total*
22-25 yr (n = 10)						
Median	14.0	23.0	35.5	20.0	15.5	43.0
Mean and SD	14.4 (8.5)	22.2 (7.5)	36.6 (12.1)	26.9 (17.9)	17.8 (10.7)	44.7 (20.8)
Range	2-32	11-31	23-63	8-68	3-43	20-85
30-33 yr (n = 12)						
Median	4.0	19.5	22.5	13.5	20.0	34.5
Mean and SD	4.8 (4.1)	19.2 (9.8)	23.9 (12.1)	13.3 (6.1)	20.2 (9.4)	33.6 (10.6)
Range	0-12	5-37	5-38	2-21	5-34	14-53
39-42 yr (n = 9)						
Median	4.0	11.0	14.0	8.0	9.0	19.0
Mean and SD	7.0 (8.8)	13.3 (13.8)	20.3 (21.7)	10.3 (8.3)	11.6 (7.8)	21.9 (14.9)
Range	0-29	0-46	4-75	3-26	1-24	4-50

We estimated the associations of age with total antral follicle number and with two subgroups defined by diameter (2 to 3.5 mm and >3.5 mm). We made the latter division because preliminary work suggested interobserver variability was less for the larger follicles; thus we were interested in checking whether associations with age held in both size groups. Ordinary least-squares linear regression was used to estimate the associations with age. The independent variable was actual age at last menstrual period. The dependent variable, follicle number, was ana-

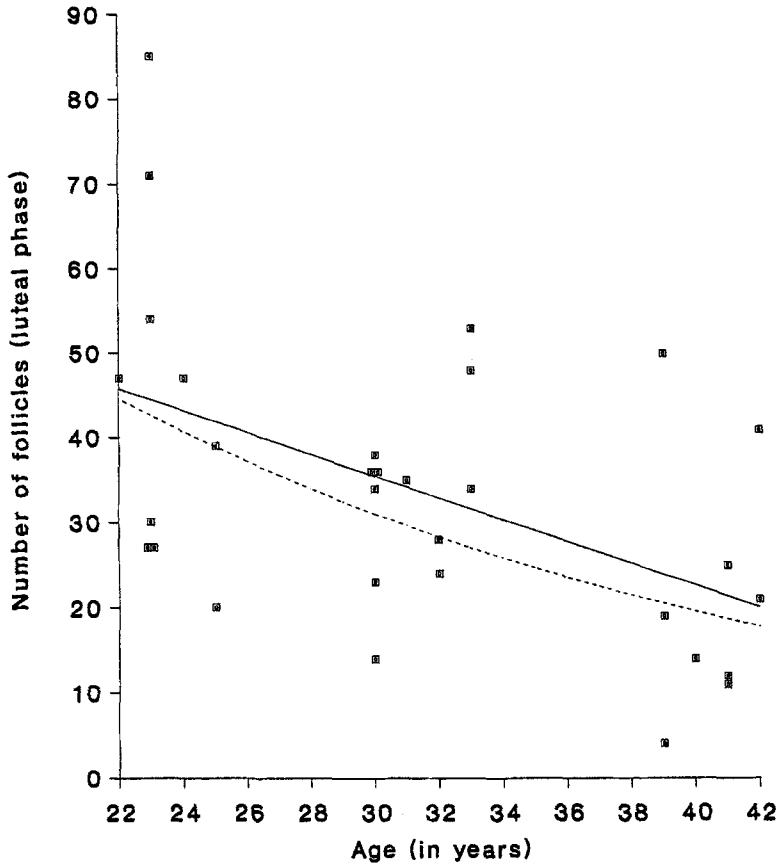

Fig. 2. Total number of follicles in luteal phase by age: observed and estimated values based on linear and logarithmic models. Linear model (——): Number of follicles = 35.5 − 1.28(Age 30), where 35.5 is estimated number of follicles at age 30. Logarithmic model (- - - -): Number of follicles = $e^{(3.47 - 0.044(\text{Age } 30))} - 1$, where $e^{3.47} - 1$ is estimated number of follicles at age 30.

lyzed in two ways: as the number of follicles per se and as a logarithmic function of the number of follicles, ln (1 + follicle number). We fitted the latter model because previous studies suggested the decline with age might be exponential.[1, 3, 5, 6] The logarithmic transformation also assists in removing skewness from the distribution of counts at any given age. Student's t statistic for the slope coefficient was used to test the significance of associations.

Results

In the follicular phase (Table I and Fig. 1) the numbers of follicles decreased with age. From the youngest to the oldest age groups median counts decreased from 35.5 to 22.5 to 14.0. The two subgroups, follicles 2 to 3.5 mm and >3.5 mm, exhibited similar declines from youngest to oldest, with the exception that similar numbers of smaller follicles were seen in the middle and oldest groups. From the linear model the estimated total counts decreased by 0.95 follicle per year of age (SE = 0.41, $p = 0.03$, $R^2 = 0.16$). From the logarithmic model we estimated that total counts declined from 39 at 20 years, to 24 at 30 years, to 14 at 40 years ($\beta = -0.048$, SE = 0.016, $p = 0.005$, $R^2 = 0.24$). In comparison with the linear model the logarithmic model predicted slightly lower counts in the middle and upper age groups. Similar results were obtained in the subgroups defined by size; for example, in the linear model the estimated decreases per year of age were 0.43 for follicles 2 to 3.5 mm (SE = 0.21) and 0.52 for follicles >3.5 mm (SE = 0.27).

Follicle counts in the luteal phase (Table I and Fig. 2) were correlated with counts in the follicular phase ($r = 0.74$) and they were higher (mean difference = 6.8, $p < 0.005$ from paired t test). As in the follicular phase the counts decreased with age. From the linear model the estimated total counts decreased by 1.3 follicles per year of age (SE = 0.41, $p = 0.004$, $R^2 = 0.25$). From the logarithmic model the estimated total counts declined from 49 at 20 years, to 31 at 30 years, to 20 at 40 years ($\beta = -0.044$, SE = 0.013, $p = 0.003$, $R^2 = 0.27$). The logarithmic model again predicted lower counts in the middle and upper age groups than were predicted by the linear model. Similar results were obtained when follicles were classified by size.

Comment

Using transvaginal ultrasonography we were able to detect an age-related decrease in the number of antral ovarian follicles. Antral follicle counts declined by about 60% between ages 22 and 42. Associations were similar in the follicular and luteal phases. Linear and logarithmic models both provided adequate fits to the data, with the latter explaining slightly more of the variance in counts.

Our data are remarkably concordant with observations from Block's autopsy series of 43 women, 25 of whom were 22 to 42 years old.[1] First, our counts of follicles ≥2 mm are close to Block's count for follicles >1 mm, a result that suggests the structures imaged by ultrasonography are indeed follicles. Second, the magnitude of the decline with age observed in our data is comparable to that observed by Block for antral follicles >1 mm. This concordance suggests that transvaginal ultrasonography provides a valid in vivo measure of the size of the antral follicle pool.

Our finding of an age-related decrease in antral follicle count is particularly intriguing when considered together with the wide variation in follicle numbers between women of about the same age found in the current study (Table I) and others.[1, 3] This suggests that counts of antral follicles may provide a biologic measure of age that is distinct from chronologic age. If so, antral follicle counts might help to predict chronologic age–related phenomena such as ease of conception, trisomy conception, and time until menopause.

The numbers of antral follicles per se may have biologic consequence. Alternatively, their relevance may be as indicators of the size of the total ovarian follicle pool. If, as has been suggested,[7] the fraction of follicles in the antral stage does not vary with the size of the total pool, then ultrasonographic counts would provide an excellent indicator of the total follicle pool. On the other hand, other data suggest that the fraction of follicles in the antral stage increases as the size of the total pool decreases.[8-10] In this case more research about the changes in the fraction would be needed before antral follicle counts could be used to estimate the size of the total pool.

From the technical perspective the counts of antral follicles will be influenced by the imaging capacities of the ultrasonography equipment and also by counting strategies. With respect to the latter several points are relevant. First, counting may be carried out during the scan, as was done in one setting,[4] or afterward, as in the current study. We found that counts made during scanning tended to be lower than counts estimated from frames of the videotape, although the correlation between measures was good ($r = 0.8$ in 31 scans also counted during scanning). Second, we anticipate that in counting from videotape the "distance" between frames (analogous to the interval between sections in histologic studies) will influence the numbers of follicles identified and the estimates of their size. We note that in our study, unlike histologic studies, the interval between videotape frames corresponded only approximately with distance; new technology that allows the probe to stay stationary while the ultrasound beam is moved through the tissue at a known velocity will improve our ability to sample the ovary at intervals that are uniformly spaced. Third, observer differences in the interpretation of which sonolucencies represent single or multiple follicles will influence counts. Because comparison with histologic sections is unlikely to be feasible, such interpretations might be standardized through the use of phantom models. In light of these sources of variation we anticipate that with current technology the "usual" range of antral follicle counts may vary between settings.

In sum, our data suggest that ultrasonographically derived counts of antral follicles provide a relatively noninvasive tool with which to study changes in the ovarian antral follicle pool and to relate these changes to reproductive and gynecologic function.

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REFERENCES

1. Block E. Quantitative morphological investigations of the follicular system in women: variations at different ages. Acta Anat 1952;14:108-23.
2. Baker TG. A quantitative and cytological study of germ cells in human ovaries. Proc R Soc Biol 1963;158:417-33.
3. 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; 7:1342-6.
4. Pache TD, Wladimiroff JW, deJong FH, Hop WC, Fauser BCJM. Growth patterns of nondominant ovarian follicles during the normal menstrual cycle. Fertil Steril 1990;54:638-42.
5. Thomford PJ, Jelovsek FR, Mattison PR. Effect of oocyte number and rate of atresia on the age of menopause. Reprod Toxicol 1987;1:41-51.
6. Kline J, Levin B. Trisomy and age at menopause: predicted associations given a link with rate of oocyte atresia. Paediatr Perinatol Epidemiol 1992;6:225-39.
7. Cran DG, Moor RM. The development of oocytes and ovarian follicles of mammals. Sci Prog 1980;66:371-83.
8. Krarup T, Pedersen T, Faber M. Regulation of oocyte growth in the mouse ovary. Nature 1969;224:187-8.
9. Gougeon A, Chainy GBN. Morphometric studies of small follicles in ovaries of women at different ages. J Reprod Fertil 1987;81:433-42.
10. Hirshfield AN. Relationship between the supply of primordial follicles and the onset of follicular growth in rats. Biol Reprod 1994;50:421-8.