BIOLOGY OF REPRODUCTION **70,** 419–424 (2004)
Published online before print 15 October 2003.
DOI 10.1095/biolreprod.103.015784

Age-Related Changes in the Ultrastructure of the Resting Follicle Pool in Human Ovaries[1]

J.P. de Bruin,[2,3] M. Dorland,[4] E.R. Spek,[5] G. Posthuma,[6] M. van Haaften,[3] C.W.N. Looman,[7] and E.R. te Velde[8]

Department of Obstetrics & Gynaecology,[3] Diakonessenhuis Utrecht, 3582 KE Utrecht, The Netherlands
Department of Human Genetics,[4] University Medical Centre Utrecht, 3584 CX Utrecht, The Netherlands
Department of Biochemistry,[5] Cell Biology and Histology, University of Utrecht, 3584 CM Utrecht, The Netherlands
Department of Cell Biology,[6] University Medical Centre Utrecht, 3584 CX Utrecht, The Netherlands
Department of Public Health,[7] Faculty of Medicine, Erasmus University Rotterdam, 3000 DR Rotterdam, The Netherlands
Department of Reproductive Medicine,[8] Division of Perinatology and Gynaecology, University Medical Centre Utrecht, 3584 CX Utrecht, The Netherlands

ABSTRACT

Age-related decline of fertility in women is the result of the decline in both quantity and quality of the resting ovarian follicle pool. The aim of the present study was to determine whether the decline of follicle quality with age is reflected by ultrastructural changes in the resting follicle pool. Ovarian biopsy specimens were obtained by laparoscopy from seven healthy women aged 25–32 yr (young group) and from 11 healthy women aged 38–45 yr (advanced-age group). A total of 182 resting follicles from the young group were compared with 81 resting follicles from the advanced-age group for signs of age-related changes by transmission-electron microscopy. The ooplasmic fraction of vacuoles was increased ($P = 0.02$), and the fraction of mitochondria decreased ($P = 0.005$), in the advanced-age group. Also, the density of the mitochondrial matrix ($P < 0.001$) and the frequency of dilated smooth endoplasmic reticulum (SER; $P = 0.001$) and Golgi complex ($P = 0.02$) were increased with age. The frequencies of ruptured mitochondrial membranes ($P = 0.001$) and dilated SER ($P = 0.003$) were increased with age in the granulosa cells. Overall follicle-quality scores, which should reflect atretic changes, were not different for the young and advanced-age groups. In conclusion, in resting follicles, the morphological changes with age are different from the changes seen in quality decline by atresia. The morphological changes with age specifically involved the mitochondria, the SER, and the Golgi complex, and they may be the cause of atresia on initiation of follicular growth because of the substantial increase in metabolic requirements.

aging, follicle, follicular development, granulosa cells

INTRODUCTION

Postponement of childbearing for socioeconomic reasons and normal age-related decline of fertility have contributed considerably to the increased incidence of subfertility [1]. Age-related decline of fertility starts at 30 yr, and the monthly probability of an ongoing pregnancy is already halved at the age of 35 yr [2]. The aging of the ovary and the concomitant decrease in both quantity and quality of the ovarian follicle pool are pivotal in the process of reproductive aging. Supportive evidence for this concept can be found in the restoration of normal pregnancy rates when older patients receive oocytes from young women in oocyte-donor programs [3].

Before birth, the complete store of follicles is already laid down in the ovaries. This store should serve the needs for reproduction for the entire life span. From birth, the follicle pool dwindles; follicles degenerate by a process called atresia either before or after they have initiated follicular growth [4]. When the follicle pool is exhausted, menopause ensues [5]. Although the decrease of follicle numbers is well established, little is known about the decrease of follicle quality with age. We know that the incidence of aneuploid oocytes increases, explaining other problems related to reproductive aging: the increasing rate of spontaneous abortions and chromosomal abnormalities in children from older mothers [6]. However, the mechanisms that bring about these changes in quality are not clear. Damage might accumulate with age, because resting follicles slumber in the ovary for decades [7]. Otherwise, follicle quality might be predetermined at the time of folliculogenesis, which is referred to as the production-line hypothesis [8]. However, evidence from animal studies testing the validity of this hypothesis is contradictory [9, 10]. We know that the proportion of resting follicles that undergo atresia is small in young women [11, 12], but we do not know how this proportion changes with age. If, in the future, we want to be able to successfully target the problems of reproductive aging, we first need to address these important questions of declining follicle quality with age.

Atresia is accompanied by specific morphological changes in the oocyte and granulosa cells that can be studied in detail by electron microscopy. In earlier work, we made an effort to design a method for scoring follicle quality in an objective fashion and to set a standard for follicle quality using morphometric data concerning the resting follicle pool in young, healthy women of proven fertility [12]. The aim of the present study was to determine whether the decrease of follicle quality with age is reflected by ultrastructural changes in resting follicles. Such changes might

[1]Supported by Serono Benelux, Abbott (unrestricted grants).
[2]Correspondence: J.P. de Bruin, Department of Reproductive Medicine, Division of Perinatology and Gynaecology, University Medical Centre, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands. FAX: 30 2505433; e-mail: jp@debruin-kers.demon.nl

Received: 23 January 2003.
First decision: 13 February 2003.
Accepted: 9 September 2003.

ISSN: 0006-3363. http://www.biolreprod.org

TABLE 1. Algorithm to calculate follicle-quality scores from morphometric data.

Morphological variable	Follicle quality score
Mitochondrial membranes, oocyte	
Intact	4.6
Hypertrophic	3.4
Ruptured	2.0
Nuclear membrane, oocyte	
Intact	0
Indented/ruptured	−0.9
SER oocyte	
Flat/associated	0
Flat/dissociated	−0.8
Dilated/associated	−0.9
Dilated/dissociated	−1.1
Mean granulosa cell nuclei (μm^2)	
$\times$ 0.1 = *a*	*a*
Oocyte nucleus (μm^2)	
$\times$ 0.003 = *b*	*b*
Proportion of granulosa cells with low-density cytoplasm	
$\times$ 1.6 = *c*	*c*
Mitochondrial matrix density oocyte	
Moderate	0
High	−0.9
Low	−0.2
	total

reveal the cellular mechanisms leading to decline of follicle quality with age and indicate the rate at which the proportion of poor-quality follicles increases with age.

MATERIALS AND METHODS

Materials

Healthy women aged 38–45 yr were recruited from patients requesting tubal sterilization. Informed consent was obtained to take small ovarian biopsy specimens during surgery. Permission was given by the local ethics committee. Women who were eligible to participate in the study had conceived within 12 mo without medical intervention in the past and still had regular menstrual cycles (cycle length, 21–35 days; maximum intercycle difference, 7 days) at the time of the present study.

Ovarian cortical biopsy specimens were taken at laparoscopy and histologically processed for transmission-electron microscopy as described elsewhere [12]. Random primordial to primary ovarian follicles were photographed for morphometric evaluation. For reasons described in our earlier work [12], we used an extensive set of morphological variables, thus generating data regarding sizes of oocytes and granulosa cells and their cell nuclei, cytoplasmic fractions of cell organelles, size distributions of mitochondria, and qualitative aspects of cell organelles of oocytes and granulosa cells. The developmental stage of the follicle was defined according to the criteria of Lintern-Moore et al. [13]: primordial (primary oocyte surrounded by a single layer of flattened granulosa cells), intermediary (primary oocyte surrounded by a single layer of flattened and cuboidal cells), and primary (primary oocyte surrounded by a single layer of cuboidal granulosa cells).

Methods of Analysis

Follicle-quality scores were calculated from morphological data using a method described in our earlier work [12]. In short, the data matrix of all morphological variables was ordered with a principal components analysis. The resulting first principal factor, a common factor that best explains the total variance of the data matrix, was assumed to reflect follicle quality. The score of individual follicles for this factor was taken to rank follicles by quality. To construct an algorithm for this overall follicle-quality score, the number of morphological variables needed to reliably provide this score was reduced by performing a stepwise forward selection. The resulting algorithm is displayed in Table 1.

The morphological data of the women in the present study (advanced-age group) were compared with similar data regarding a group of healthy women aged 26–32 yr (young group) from our earlier study [12]. Group differences for morphological data and follicle-quality scores were tested by linear regression in a restricted maximum likelihood (REML) variance components analysis [14] or by logistic regression in a generalized linear mixed model analysis [15] as appropriate. To account for the multilevel design of the present study (studied subjects are taken to be a random sample of all possible women, and within the studied subjects, the studied follicles are taken to be a random sample of all possible follicles), subject number and follicle number were included as random factor in both analyses. In this way, the bias caused by different numbers of assessed follicles per woman is reduced, and significance values are adjusted for the dependency of observations within women. Outcomes of the REML variance components analysis are the estimated means for both groups, the SEM for the difference between these means, and the significance value. Outcomes of the generalized linear mixed model analysis are the estimated means for both groups, the odds ratio for the difference between these means, the 95% confidence interval (CI) for the odds ratio, and the significance value.

Analyses were carried out with Genstat 5 (release 3.1; Lawes Agricultural Trust, Rothamsted, U.K.).

TABLE 2. Ages of subjects and number of assessed ovarian follicles.

Young group			Advanced-age group		
Subject	Age (y)	Follicles (n)	Subject	Age (y)	Follicles (n)
I-1	32	27	II-1	40	3
I-2	32	15	II-2	40	2
I-3	32	18	II-3	45	7
I-4	26	18	II-4	40	5
I-5	29	34	II-5	39	10
I-6	31	51	II-6	43	4
I-7	32	19	II-7	39	17
			II-8	41	4
			II-9	40	10
			II-10	41	1
			II-11	41	18
Mean	30.6	26.0		40.8	7.4
Total		182			81

RESULTS

The young group consisted of seven women with a mean age of 30.6 yr. The birth of the last child in this group occurred at a mean age of 28.9 yr (range, 25–32 year). The advanced-age group consisted of 11 women with a mean age of 40.8 yr. The birth of the last child in this group occurred at a mean age of 32.4 yr (range, 27–36 yr). A total of 182 follicles were assessed by electron microscopy in the young group and a total of 81 follicles in the advanced-age group (Table 2). The outcomes of morphological variables for both groups are listed in Table 3.

In the ooplasma lipid droplets, multivesicular bodies and the Golgi complex only made up a small fraction in both the young and advanced-age groups. A significant increase of the ooplasmic fraction of vacuoles in the advanced-age group compared to the young group could be noted (5.2%, SEM = 2.4%, $P = 0.02$). The fraction of the ooplasma taken up by mitochondria was significantly reduced by 1.9% in the advanced-age group (SEM = 0.7%, $P = 0.005$). The mean profile areas of individual mitochondria were comparable for the young and advanced-age groups.

In the oocytes, the aspect of the nuclear membrane, the mitochondrial membranes, and the number of microvilli were comparable for the young and advanced-age groups. The frequency of high density of the mitochondrial matrix was significantly increased in the advanced-age group (odds ratio = 3.0, CI = 1.6–5.6, $P < 0.001$). The frequency of flat and associated smooth endoplasmic reticulum (SER) showed a significant decrease, and of dilated and dissociated SER an increase, in the advanced-age group (odds ratio = 1.7, CI = 1.2–2.5, $P = 0.001$). Furthermore, a sig-

TABLE 3. Outcomes for morphological variables of follicle quality and development.

	Young group		Advanced-age group	
Variables	Mean	SD	Mean	SD
Oocytes				
Mean area, oocyte	850 μm^2	244.0	927 μm^2	258
Mean area, oocyte nucleus	199 μm^2	97.7	197 μm^2	94.0
Mean nucleus : oocyte ratio	0.23 μm^2	0.09	0.21 μm^2	0.08
Cytoplasmic fraction				
Lipid droplets	0.23%	0.55	0.11%	0.17
Multivesicular bodies	2.25%	2.99	2.28%	2.77
Vacuoles	1.74%	4.86	6.98%[a]	12.4
Golgi complex	0.27%	0.35	0.21%	0.21
Mitochondria	7.14%	2.77	5.22%[a]	2.41
Mean profile area, mitochondria	0.29 μm^2	0.16	0.28 μm^2	0.15
Nuclear membrane				
Intact	67.6%		70.4%	
Indented or ruptured	32.4%		29.6%	
Mitochondrial membranes				
Intact, parallel, or transverse cristae	91.8%		90.1%	
Intact, arch-like cristae	2.7%		3.7%	
Ruptured	5.5%		6.2%	
Mitochondrial matrix density				
Low	27.5%		14.8%	
Moderate	63.7%		60.5%	
High	8.8%		24.7%[a]	
SER				
Flat/associated	65.9%		42.0%[a]	
Flat/dissociated	9.9%		9.9%	
Dilated/associated	15.9%		23.5%	
Dilated/dissociated	8.2%		24.7%[a]	
Number of microvilli				
Low	62.6%		66.7%	
High	37.4%		33.3%	
Golgi complex				
None present	25.3%		24.7%	
Flat	57.1%		43.2%	
Dilated	17.6%		32.1%[a]	
Granulosa cells				
Mean area, granulosa cells	30.5 μm^2	14.3	31.5 μm^2	14.4
Mean area, granulosa cell nuclei	13.0 μm^2	7.71	13.4 μm^2	7.66
Mean nucleus : granulosa ratio	0.42 μm^2	0.16	0.42 μm^2	0.15
Cells with low-density chromatin	79.7%		79.4%	
Cells with low-density cytoplasm	77.9%		69.4%	
Cells with intact mitochondria	96.8%		91.8%[a]	
Cells with flat endoplasmic reticulum	88.5%		62.0%[a]	
Development				
Follicle stage				
Primordial	31.5%		31.2%	
Intermediary	54.1%		63.6%	
Primary	14.4%		5.5%	
Zona pellucida				
Absent	87.5%		85.2%	
Partially developed	11.8%		13.6%	
Complete	0.8%		1.2%	
Number of zonula adherentia	2.69	2.28	3.37	2.82

[a] $P \leq 0.05$ versus young group.

nificant increase of dilation of the Golgi complex was found in the advanced-age group (odds ratio = 2.3, CI = 1.2–4.5, $P = 0.02$).

In the granulosa cells, the proportion of cells with low condensation of chromatin and low density of cytoplasm were comparable for the young and advanced-age groups. The frequency of granulosa cells with ruptured mitochondrial membranes was significantly increased in the advanced-age group (odds ratio = 5.0, CI = 1.9–12.9, $P = 0.001$). As in the oocytes, the frequency of dilation of the endoplasmic reticulum in granulosa cells was significantly elevated in the advanced-age group (odds ratio = 3.2, CI = 1.5–6.9, $P = 0.003$).

The distributions of follicle stages were not different for

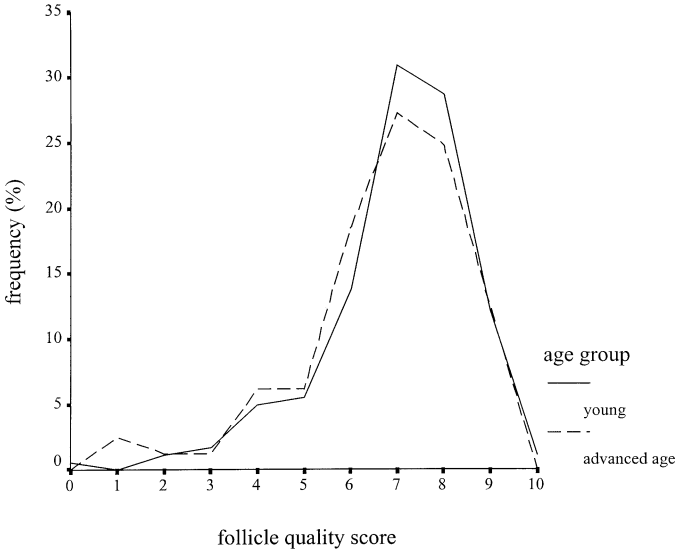

FIG. 1. Distribution of follicle-quality scores for women aged 25–32 yr (young group) and for women aged 38–45 yr (advanced-age group). Scale 0–10 = poor–good quality.

the young and advanced-age groups. Also, no differences were found for the aspect of the zona pellucida and the numbers of zonula adherentia between the two age groups.

The distributions of follicle-quality scores for the advanced-age and young groups are displayed in Figure 1. A large overlap between the groups existed, but toward lower-quality scores, a higher frequency for the advanced-age group could be noted. The mean follicle score was 0.35 points lower in the advanced-age group, which did not reach statistical significance (SEM = 0.31, $P = 0.26$). Figure 2 includes micrographs with examples of morphological changes in follicles with age.

DISCUSSION

All subjects in the present study were healthy women with regular menstrual cycles and proven fertility. Any changes observed in the morphology of resting follicles between the young and advanced-age groups therefore likely are related to age and not to other mechanisms. Considering the ages of the groups, the women of the young group are not yet expected to show age-related decline of fertility, whereas the women in the advanced-age group are expected to show a severe reduction of fertility or even be infertile [2]. The women in the advanced-age group, not surprisingly, gave birth to their last child at a significantly higher mean age than the women in the young group. Therefore, theoretically, it cannot be entirely ruled out that they slightly differ from the young group in fertility potential and constitution of their follicle pool.

The distribution of follicle stages did not change with age in the present study. Others have reported a very moderate decrease in the proportion of primordial follicles and an increase in the proportion of intermediary and primary follicles from 30 to 40 yr of age [16]. As in our earlier work, no apparent correlation was found between follicle stage and morphological signs of atresia [12]. We did find a significant increase in the cytoplasmic fraction of vacuoles in oocytes with age, which confirms earlier findings in aging oocytes both in vivo and in vitro [17, 18]. No increase of the fractions of multivesicular bodies and lipid droplets in oocytes with age was found, which confirms the findings of other investigators [17, 18].

For the mitochondria, a significant decrease of their cytoplasmic fraction in the oocytes was found with age, whereas the mean profile area of the individual mitochondria did not change. Hence, we conclude that in the oocytes, destruction of mitochondria takes place with increasing age. To our knowledge, no comparable studies concerning age-related changes of mitochondria in resting follicles exist. Mitochondria in preovulatory follicles appear to increase in number and size with age [19]. Increased sizes of mitochondria in combination with changes in mitochondrial membrane potential are associated with increased oxidative stress [20]. We also found a significant increase in the proportion of oocytes with increased density of the mitochondrial matrix with age, which is in line with findings for aged oocytes in vitro [18]. In the granulosa cells, mitochondrial damage with age was indicated by a significant increase in the proportion of granulosa cells with ruptured mitochondrial membranes. We did not quantify the fraction of mitochondria in these cells, but destruction of mitochondria in the granulosa cells seems most likely.

A significant increase in the proportion of oocytes showing dilation of the SER and Golgi complex was found, which to our knowledge has not been reported earlier in relation to aging, only in relation to atresia [18, 21]. Similar to findings in mitochondria, increasing oxidative stress with age probably induces changes in the membrane potential of SER and Golgi complex, which causes the observed dilation of these organelles.

Follicle-quality scores were calculated according to a method previously described [12]. This score is designed to accurately reflect the outcomes of all morphological variables with respect to atresia. The mean follicle-quality score was not significantly reduced in the advanced-age group compared to the young group, although a number of individual morphological variables showed significant age-related changes. Note that only two of these variables are included in the algorithm for the follicle-quality score (mitochondrial matrix density and aspect of the SER in the oocyte), with a maximal contribution of −1.8 points to the total. On the basis of our findings in young women, it can be concluded that the morphological changes that occur with atresia in resting follicles are different from the morphological changes that occur with aging. Although the proportion of follicles showing atresia apparently does not change with age, the changes in mitochondria, SER, and Golgi complex as well as the increased vacuolarization of the ooplasm probably indicate the accumulation of damage in resting follicles with age.

The subtlety of the observed changes in the morphology of resting follicles with age may not seem to be in proportion to the expected decline of fertility from 30 to 40 yr of age. However, these changes might exert a significant effect on fertility in a number of ways. First, because of a low metabolic rate [22], the detrimental effect of damaged mitochondria, SER, and Golgi complex might be limited in resting follicles. However, once rapid follicular growth is initiated and metabolic and synthetic rates should increase, the cell organelles might fall short in meeting these requirements, inducing quality decline and, eventually, atresia. Oocytes with low metabolic activity become embryos that develop poorly and have low implantation rates [23]. Furthermore, an association between compromised metabolism and errors at meiotic recombination in mouse models has been reported [24]. Second, follicles that become atretic because of age-related damage might disappear quickly, resulting in decreasing resting follicle numbers rather than an

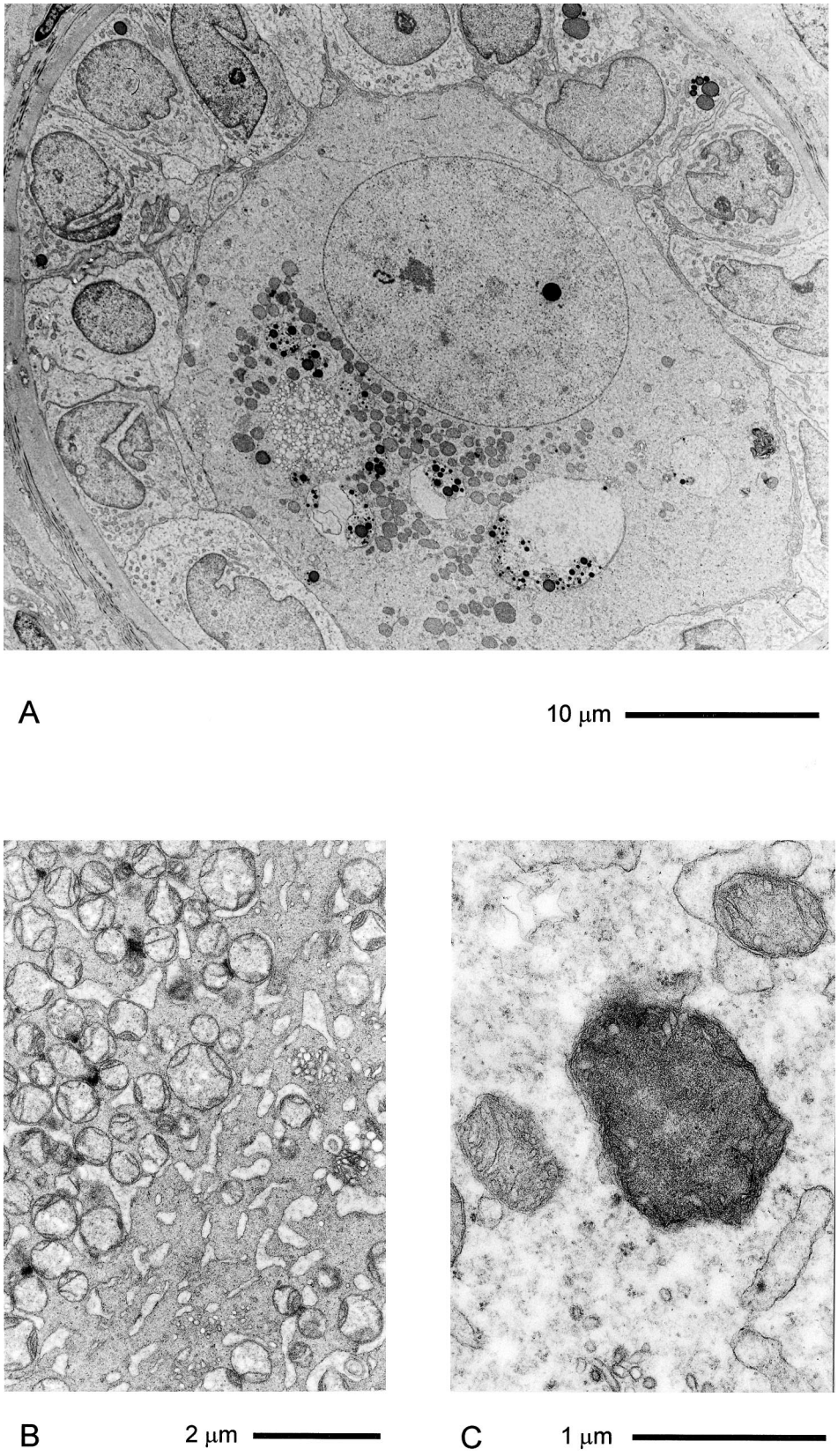

FIG. 2. Examples of morphological changes in follicles from women of advanced reproductive age. **A**) Primary follicle with vacuoles of varying sizes in the ooplasma. **B**) Detail of ooplasma with arch-like mitochondria and dilated SER. **C**) Detail of ooplasma showing mitochondria with high density of the mitochondrial matrix.

increasing proportion of poor-quality follicles. Thus, atresia would pass largely unnoticed, and the rate of atresia would be underestimated. Third, the observed age-related changes may be associated with changes in parameters other than those investigated during the present study that might also contribute to fertility decline with age. For example, we did not investigate the genetic material of the oocytes, but others have found a correlation between age and the accumulation of damage in the chromosomes and the meiotic spindle [25, 26]. Finally, if we draw a comparison to other long-lived cells (e.g., nerve cells), the findings are similar to those in oocytes and granulosa cells. In neurons of pri-

mate brains, only limited morphological changes are observed with increasing age, such as lipofuscin accumulation and folding of membranes. However, these limited changes are correlated with a significant negative effect on cognitive functions [27].

In conclusion, the age-related changes in the mitochondria, SER, and Golgi complex suggest a role for oxidative damage. These changes had characteristics other than those observed in the atretic process of resting follicles in young women, explaining why overall follicle-quality scores, which should indicate quality decline by atresia, did not significantly change with age. Consequently, the rate of atresia in resting follicles does not seem to increase with increasing age. However, the subtle changes that indicate age-related cellular damage might induce quality decline and atresia after the initiation of follicular growth, when metabolic and synthetic requirements increase. This cellular mechanism for the qualitative decline of follicles, together with rapidly declining follicle numbers, may be responsible for declining fertility in the fourth decade of life in women. It may be worthwhile to evaluate this hypothesis further by ultrastructural studies of growing oocytes.

REFERENCES

1. Mosher WD, Pratt WF. Fecundity and infertility in the United States: incidence and trends. Fertil Steril 1991; 56:192–193.
2. 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. Br Med J 1991; 302:1361–1365.
3. Navot D, Bergh PA, Williams MA, Garrisi GJ, Guzman I, Sandler B, Grunfeld L. Poor oocyte quality rather than implantation failure as a cause of age-related decline in female fertility. Lancet 1991; 337: 1375–1377.
4. Block E. A quantitative morphological investigation of the follicular system in newborn female infants. Acta Anat 1953; 17:201–206.
5. 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.
6. Te Velde ER, Pearson PL. The variability of female reproductive aging. Hum Reprod Update 2002; 8:141–154.
7. Gosden RG. Oocyte development throughout life. In: Grudzinskas JG, Yovich JL (eds.), Cambridge Reviews in Human Reproduction, Gametes—The Oocyte. Cambridge, U.K.: Cambridge University Press, 1995:119–149.
8. Henderson SA, Edwards RG. Chiasma frequency and maternal age in mammals. Nature 1968; 218:22–28.
9. Hirshfield AN. Heterogeneity of cell populations that contribute to the formation of primordial follicles in rats. Biol Reprod 1992; 47:466–472.
10. Meredith S, Doolin D. Timing of activation of primordial follicles in mature rats is only slightly affected by fetal stage at meiotic arrest. Biol Reprod 1997; 57(July):63–67.
11. Faddy MJ, Gosden RG. A mathematical model of follicle dynamics in the human. Hum Reprod 1995; 10:770–775.
12. De Bruin JP, Dorland M, Spek E, Posthuma G, van Haaften M, Looman CWN, te Velde ER. Ultrastructure of the resting follicle pool in the ovaries of healthy young females. Biol Reprod 2002; 66:1151–1160.
13. Lintern-Moore S, Peters H, Moore GPM, Faber M. Follicular development in the infant human ovary. J Reprod Fertil 1974; 39:53–64.
14. Searle SR, Casella G, McCulloch CE. Variance Components. New York: John Wiley & Sons; 1992.
15. Schall R. Estimation in generalized linear models with random effects. Biometrica 1991; 78:719–727.
16. Gougeon A, Chainy GBN. Morphometric studies of small follicles in ovaries of women at different ages. J Reprod Fertil 1987; 81:433–442.
17. Guraya SS. Correlation between the findings of light and electron microscopy in human primordial follicles. Acta Anat 1970; 77:617–635.
18. Sathananthan AH. Ultrastructure of the human egg. Hum Cell 1997; 10:21–38.
19. Muller-Hocker J, Schafer S, Weis S, Munscher Ch, Strowitzki Th. Morphological-cytochemical and molecular genetic analysis of mitochondria in isolated human oocytes in the reproductive age. Mol Hum Reprod 1996; 2:951–958.
20. Sastre J, Pallardo FV, Garcia de la Asuncion J, Vina J. Mitochondria, oxidative stress and aging. Free Radic Res 2000; 32:189–198.
21. Stankova J, Cech S. Ultrastructural changes during atresia of human ovarian follicles: I. primordial follicles. Z Mikrosk Anat Forsch 1983; 97:915–928.
22. Oktay K, Nugent D, Newton H, Salha O, Chatterjee P, Gosden RG. Isolation and characterization of primordial follicles from fresh and cryopreserved human ovarian tissue. Fertil Steril 1997; 67:481–486.
23. Van Blerkom J, Davis PW, Lee J. ATP content of human oocytes and developmental potential and outcome after in-vitro fertilization and embryo transfer. Hum Reprod 1995; 10:414–424.
24. Eichenlaub-Ritter U. Genetics of oocyte aging. Maturitas 1998; 30: 143–169.
25. Lim AS, Tsakok MF. Age-related decline in fertility: a link to degenerative oocytes? Fertil Steril 1997; 68:265–271.
26. Battaglia DE, Goodin P, Klein NA, Soules MR. Influence of maternal age on meiotic spindle assembly. Hum Reprod 1996; 11:2217–2222.
27. Peters A, Leahu D, Moss MB, McNally KJ. The effects of aging on area 46 of the frontal cortex of the rhesus monkey. Cereb Cortex 1994; 4:621–635.