Age-related variations in follicular apolipoproteins may influence human oocyte maturation and fertility potential

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Objective: To investigate involvement of specific apolipoproteins in the process of human oocyte maturation and age-related infertility as molecular constituents of follicular fluid.
Design: Laboratory-based observational study.
Setting: Basic science laboratory at a large academic institution.
Patient(s): Follicular fluid obtained from healthy women aged 18 to 45 years undergoing in vitro fertilization for unexplained infertility, ovulatory dysfunction, tubal disease, male factor infertility, or oocyte donation.
Intervention(s): None.
Main Outcome Measure(s): Specific concentration of apolipoproteins and content of lipoprotein particles in follicular fluid and blood plasma as related to reproductive aging.
Result(s): We registered a decline of follicular apolipoprotein A1 (Apo A1) and apolipoprotein CII (Apo CII) and an increase of the apolipoprotein E (Apo E) with age, which parallels a lower number of retrieved mature oocytes in older women. Follicular apolipoprotein A1, apolipoprotein B (Apo B), apolipoprotein E, and apolipoprotein C II are present in diverse heterogeneous complexes including very-low-density lipoproteins (VLDL), intermediate-low-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) that vary with patient age and differ from the blood plasma lipoprotein complexes.
Conclusion(s): Age-related variation in follicular apolipoprotein content and distribution in the cholesterol particles may be associated with the decrease in production of mature oocytes and the age-related decline in fertility potential. (Fertil Steril® 2010;93:2354–61. ©2010 by American Society for Reproductive Medicine.)

Key Words: Apolipoprotein, lipoprotein particles, VLDL, IDL, LDL, HDL, human follicular fluid, in vitro fertilization, human oocyte maturation

The human female reproductive aging process is marked by a sharp decline in ovarian germ cell supply, ultimately leading to ovarian reproductive failure (1). The mechanisms that lead to this decline are poorly understood.

Follicular fluid (FF), produced by granulosa cell secretion and by diffusion from the theca capillaries, contains over 200 different proteins. It has been proposed that many of these proteins play an important role in the maturation of oocytes and assist in the protection of the follicle from toxic injury and oxidative stress as well as in lipid transport (2).

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Apolipoproteins have a well-known role in cholesterol and triglyceride trafficking. Variation in serum levels of lipoproteins in women with polycystic ovarian syndrome and ovarian cancer have been identified and may be biomarkers for adverse health outcomes (3, 4). In addition, some apolipoproteins have been identified in FF and are thought to play a direct role in reproduction. Previous investigations have demonstrated that high-density lipoproteins (HDL) carrying sphingosine 1-phosphate in FF and may play a role in the development of the follicle by stimulating angiogenesis (5). More recently, sphingosine-1-phosphate associated with higher density HDL particles has been shown to have an anti-apoptotic effect systemically (6). The sterility of HDL receptor-negative (SR-BI$^{-/-}$) female mice suggests a link between female infertility and molecular HDL integrity (7). Low-density lipoproteins (LDL) had been reported to play a role in estradiol synthesis in bovine ovarian follicles (8). The presence of the other lipoprotein complexes, the very-low-density lipoproteins (VLDL) and intermediate low-density (IDL) complexes, in mammalian FF has not been reported in the literature. Although hormonal levels in FF have been previously studied and found to differ with aging (9), other

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proteins, specifically lipoproteins, have not been systematically identified to vary in relation to human aging and reproductive potential. Apo A1, Apo B, Apo E, and Apo CII are known to be present in different protein and lipid-protein complexes (10, 11). The complexation status of these apolipoproteins in human FF and the association with reproductive aging are presently unknown.

We investigated the FF protein composition including apolipoproteins and assessed for variation in relation to reproductive aging. We hypothesized that FF protein composition, including the family of the apolipoproteins, varies with reproductive aging and that such variations in FF parallel the age-related decline in fertility potential and production of mature oocytes.

MATERIALS AND METHODS

Study Population

With approval by the Beth Israel Deaconess Medical Center institutional review board, eligible women undergoing in vitro fertilization (IVF) were identified for enrollment on the day of oocyte retrieval. Inclusion criteria included healthy women, age 18 to 45 years, undergoing oocyte retrieval for oocyte donation, unexplained infertility, ovulatory dysfunction, tubal disease, or male factor infertility. After their written informed consent was obtained, the participants completed a brief health and reproductive history questionnaire. We enrolled 22 young and 16 older women. Multiple demographic and clinical variables were obtained including cycle number, body mass index (BMI), cycle day 3 follicle stimulating hormone (FSH) level, and total dose of gonadotropins used.

Stimulation and Follicular Fluid Retrieval

The stimulation protocol was standard and included down-regulation with either a gonadotropin-releasing hormone (GnRH) agonist or antagonist and hyperstimulation with recombinant follicle stimulating hormone (FSH). Human chorionic gonadotropin (hCG) was given when at least three follicles $\geq$18 mm were present on vaginal ultrasound, and vaginal oocyte retrieval was performed 36 hours later. During the procedure, an ultrasound was used to measure all follicles in two dimensions before aspiration and collection. A follicle was labeled "lead" and used in the experiments if the mean diameter was $\geq$16 mm.

The FF aliquots were then examined by an embryologist to detect and remove cumulus–oocyte complexes. Although all follicles were aspirated and oocytes retrieved from these follicles, the fluid from two to three lead follicles was pooled into one vial and used for the analyses. The blood-free FF was centrifuged at 13,000 rpm for 20 minutes to separate cells, debris, and FF. Blood collected on the day of the oocyte retrieval was mixed with 10 μL 0.5 mM ethylenediaminetetraacetic acid (EDTA) and centrifuged at 13,000 rpm for 20 minutes. Plasma was isolated after centrifugation, aliquoted, and stored at –80°C if not immediately analyzed. Samples from a total of 38 patients were analyzed.

2D-PAGE

Before we performed two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), the FF was subjected to protein A G sepharose chromatography (Pierce, Rockford, IL) to remove the majority of immunoglobulins. The remaining fluid was treated with 1% trichloroacetic acid (TCA) and the precipitated proteins were subjected to 2D-PAGE, which was performed in duplicate on all samples individually. We used 70 μg of protein for 2D-PAGE separation. For the first dimension separation, a pH 3.0 to 10.0 ampholine gradient was used according to the supplier's protocol (Amersham Biosciences, Uppsala, Sweden). A sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) acrylamide gradient gel (4% to 20%) was used for the second dimensional separation. After separation in SDS-PAGE, the proteins on the gel were fixed and visualized by Coomassie or silver staining using a Silver Stain Plus Kit (Bio-Rad Laboratories, Hercules, CA).

Mass Spectrometry Analysis and Protein Identification

Spots of interest were excised from the silver-stained gel and then digested with trypsin as previously described elsewhere (12, 13). Digested peptides were extracted twice from gel pieces with 50 μL of 50% acetonitrile and 0.1% trifluoroacetic acid. The pooled supernatants were dried in a SpeedVac (Savant Instruments, Holbrook, NY), and the peptides were dissolved in 10 uL of 50% acetonitrile with 0.1% trifluoroacetic acid. Peptide fragments were characterized based on their molecular mass. Mass spectroscopy was performed on an Ultraflex time-of-flight instrument (Bruker Daltonics, Billerica, MA). Mass spectra were calibrated with angiotensin II (1046.54 d), angiotensin I (1296.68 d), substance P (1347.74 d), bombesin (1619.82 d), adrenocorticotropic hormone (ACTH18-39) (2465.20 d), and insulin (5733.54 d).

Western Blot and ELISA

Individual apolipoproteins in FF (50 μg protein) were visualized by Western blot with different protein specific antibodies: Apo A1 (178422; Calbiochem, San Diego, CA), Apo B (ab13993; Abcam, Cambridge, MA), Apo CII (178428; Calbiochem), and Apo E (0650-1904; AbD Serotec, Raleigh, NC). The Apo A1 and Apo B content in individual FF was measured by enzyme-linked immunosorbent assay (ELISA) with a protein-specific kit (coefficient of variation 6% to 10%) according to the supplier's protocol (ALerCHEK, Portland, ME). All samples were measured in duplicate. Where kits were not commercially available, the protein content was evaluated by pixel counting of the protein-specific immunoreactive Western blot bands in ImageJ software (U.S. National Institutes of Heath, http://rsb.info.nih.gov/ij/). The Western blot data in the figures includes only the gel

portion corresponding to the protein-specific immunologic reactions.

Isolation of VLDL, IDL, LDL and HDL

Follicular fluid or blood plasma (0.4 mL), overlaid with KBr solution, was centrifuged at 100,000 rpm. Four centrifugations were performed for each sample, as specified in the procedure for isolating VLDL, IDL, LDL, and HDL complexes (14). Isolation was performed with the FF of five young women (aged 24 to 28 years) and four older women (aged 41 to 43 years) and the blood plasma from three of the younger and three of the older women.

High-Performance Liquid Chromatography (HPLC) and Light Scattering

Individual FF samples were fractionated by size exclusion chromatography (SEC) on a Superose 6 column and by SMART HPLC (Pharmacia, Sweden), as previously described elsewhere (15). Individual fractions were next analyzed by Western blot or ELISA in duplicate for presence and content of specific proteins. The hydrodynamic radius of the molecules and their complexes was determined by light scattering (LS) according to the supplier's protocol (Wyatt Technology, Santa Barbara, CA).

Statistical Analysis

Data analysis was conducted using the Statistical Analysis System 9.1.3 software (SAS Institute, Cary, NC). The analyses presented here include only women <35 years (reproductively young) and ≥ 40 years (reproductively older). We stratified by age because age is considered one of the strongest predictors of IVF outcome (16). Furthermore, the cutoffs used in this study correspond to the youngest and oldest age categories used by the Centers for Disease Control and Prevention and the Society for Assisted Reproductive Technology (17). To characterize the fertility potential of younger and older women we calculated the mean ($\pm$ standard deviation) and proportions for several cycle characteristics. We compared these characteristics between the two age strata with a Mann-Whitney *U* test or chi-square test, as appropriate. Remaining results are presented as the mean ($\pm$ standard error), and age groups were compared with the Mann-Whitney *U* test. $P<.05$ was considered statistically significant.

RESULTS

Changes in FF Peptide Composition with Age

We compared protein composition profiles from pooled lead follicles with 2D-PAGE. Two peptide spots were identified as having consistently higher spot volume in younger compared with older FF (Fig. 1A). Applying Edman degradation, HPLC, and mass spectrometry (not shown), both spots were identified as Apo A1. The intensity of the spot corresponding to the previously identified protein transthyretin (TTR) (2) remained age-independent and was used as an internal standard for equal protein loading.

Two different assays were used to verify the results of the 2D-PAGE experiments. Apo A1 was visualized in FF from individual women by Western blot with an Apo A1-specific antibody. The Western blot analysis of FF supported the 2D-PAGE observation that FF from younger women (see Fig. 1B, lane 1) appears to have a larger Apo A1 content than FF from older women (see Fig. 1B, lane 2). The ELISA assay revealed a similar tendency: a higher Apo A1 content among younger (9.6 ± 0.7 mg/mL) compared with older (8.3 ± 0.7 mg/mL) women, although the difference in this assay was not statistically significant ($P=.33$) (see Fig. 1B).

Because Apo A1 is known to colocalize with Apo E in blood plasma and Apo E presence in FF has been reported (18), we compared Apo E levels in FF by Western blot. As measured by pixel count in Figure 1C, the average Apo E content was higher in older women than in young women ($P=.008$). This result is supported by the 2D-PAGE observation (see Fig. 1A).

The 2D-PAGE experiments (see Fig. 1A) reveal Apo CII presence as well, mainly in FF from younger patients. Again, the concentration in FF was assessed by Western blot (see Fig. 1D). As measured by pixel count, the average Apo CII content is higher in young women than in older women ($P=.008$).

Apo B and Apo E in FF Apo A1 as Several Complexes That Vary in Size with Age

A representative FF separation profile from a 24-year-old patient is shown in Figure 2A. The general molar mass and size distribution profile was similar in both young and older FF (data not shown). Equal volumes of the eluted SEC-LS fractions were next analyzed for Apo A1 and Apo B content by ELISA. Western blot with protein-specific antibodies was used to determine the Apo E and Apo CII content. By combining the LS profile and the ELISA results for the individual SEC-LS fractions, Apo A1 was found to elute reproducibly in several complexes with diverse molecular mass (see Fig. 2B). In the FF of young women, nearly half of Apo A1 was found in fraction 11, corresponding to a complex with molecular mass of $\sim$451 kd (27 nm), while in older women less than 20% of Apo A1 eluted in fraction 11. Among older women, approximately 30% was found in fraction 15, with apparent molecular mass of $\sim$213 kd, compared with less than 10% in younger women. Although most Apo B ($>85\%$) in both younger and older women was found in large complexes with molecular mass of 17–19 md (see Fig. 2C, fractions 3–7), the concentrations appear to be substantially higher in older than in younger women.

The Western blot results with Apo E–specific antibodies demonstrated that a majority (80%) of Apo E in the FF of young women eluted in the 370–213 kd molecular mass fractions (see Fig. 2D, fractions 13–15). In the FF of older

FIGURE 1

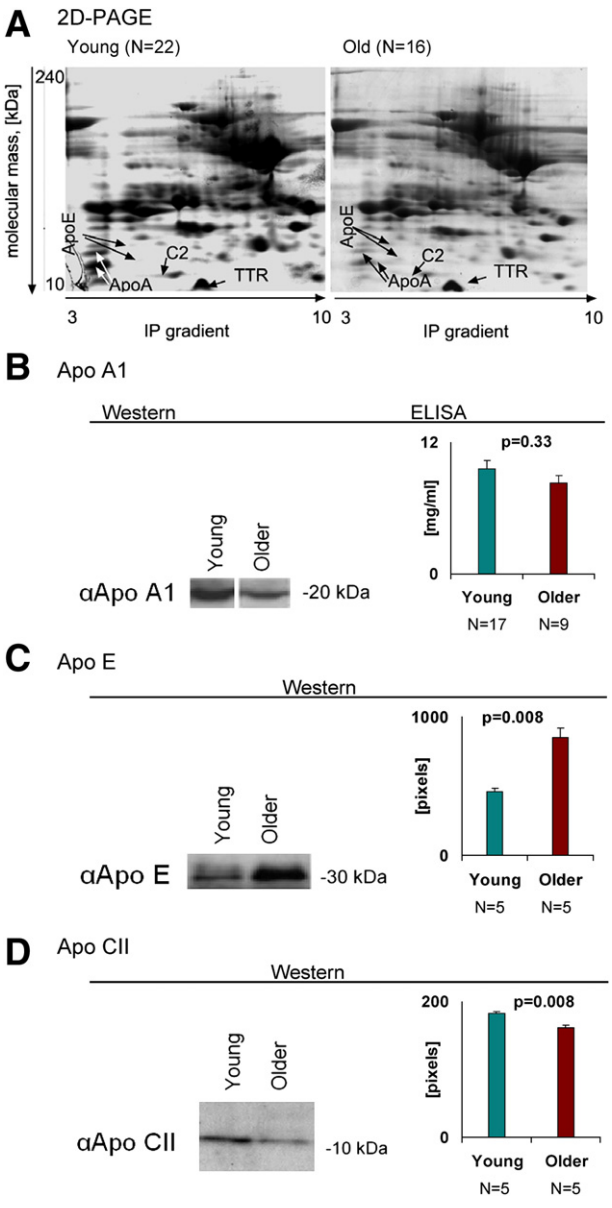

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FIGURE 1 Continued

Age-related changes in apolipoprotein content in follicular fluid from lead follicles of young and older women. Figures shown by 2D-PAGE and Western blot are representative for each age group. The intensity of the immunologic reaction bands specific to Apo E and Apo CII is measured in pixels by scanning and is graphically presented at the right of the Western blot. (**A**) Follicular fluid (FF) was individually collected from 22 young and 16 older women. The age group of the sample is indicated on each figure. The isoelectric points (IP) gradient distribution is shown at the bottom. The molecular mass gradient separation in the second dimension is shown in kilodaltons (kd). The identity of the Apo A1 spots is determined by LC-MS/MS and shown with arrows. Apo E and transthyretin (TTR) were determined based on migration, IP, and molecular mass. (**B**) FF is separated by SDS-PAGE and visualized by Western blot with an Apo A1–specific antibody. The Apo A1 protein amount (mg/mL) is determined by enzyme-linked immunosorbent assay (ELISA). The ELISA results are illustrated by histogram in which the bars represent the quantity of Apo A1. (**C**) Western blot with Apo E–specific antibody in FF. (**D**) Western blot with Apo CII–specific antibody in FF.

women, however, part of Apo E (>20%) was consistently found also in smaller ~140 kd complexes (see Fig. 2D, fraction 17). Finally, independent of age, Apo CII was exclusively found in a fraction corresponding to complexes with molecular mass of ~213–140 kd (see Fig. 2E, fractions 15–17).

Thus, in human FF, Apo A1, Apo B, Apo CII, and Apo E are organized in several complexes with sizes ranging from 19 md to 140 kd. Furthermore, the distribution of Apo A1, Apo B, and Apo E in these complexes appears to vary with age.

Apo A1, Apo B, and Apo E Constituents of Follicular Lipoprotein Cholesterol HDL, LDL, and IDL Particles

Follicular Apo A1 was detected by ELISA mainly in association with HDL and LDL (Fig. 3A). However, there were no statistically significant age-related differences (all P>.5). Results were similar for the distribution of Apo A1 in blood plasma complexes (all P>.1), but the overall Apo A1 concentration in blood plasma was greater for all cholesterol particles compared with FF (see Fig. 3A).

Similar to the known blood plasma distribution, follicular Apo B concentration was highest in the LDL fraction (see Fig. 3B). The mean Apo B level in blood plasma was consistently higher, albeit not statistically significant (all *P*>.2), in younger compared with older women for all cholesterol complexes.

The Western blot analyses revealed that the majority of the follicular Apo E is in the HDL fraction (see Fig. 3C). The pixel intensity of the Apo E-specific band in HDL was higher in younger compared with older women (*P*=.008). In addition, a substantial proportion of total Apo E was found in the follicular LDL fraction but only minimal amounts in VLDL and IDL for both young and older women. In blood plasma, however, Apo E was present in relatively large quantities in all of the lipoprotein fractions. The concentration of Apo E was greater in older women compared with younger

FIGURE 2

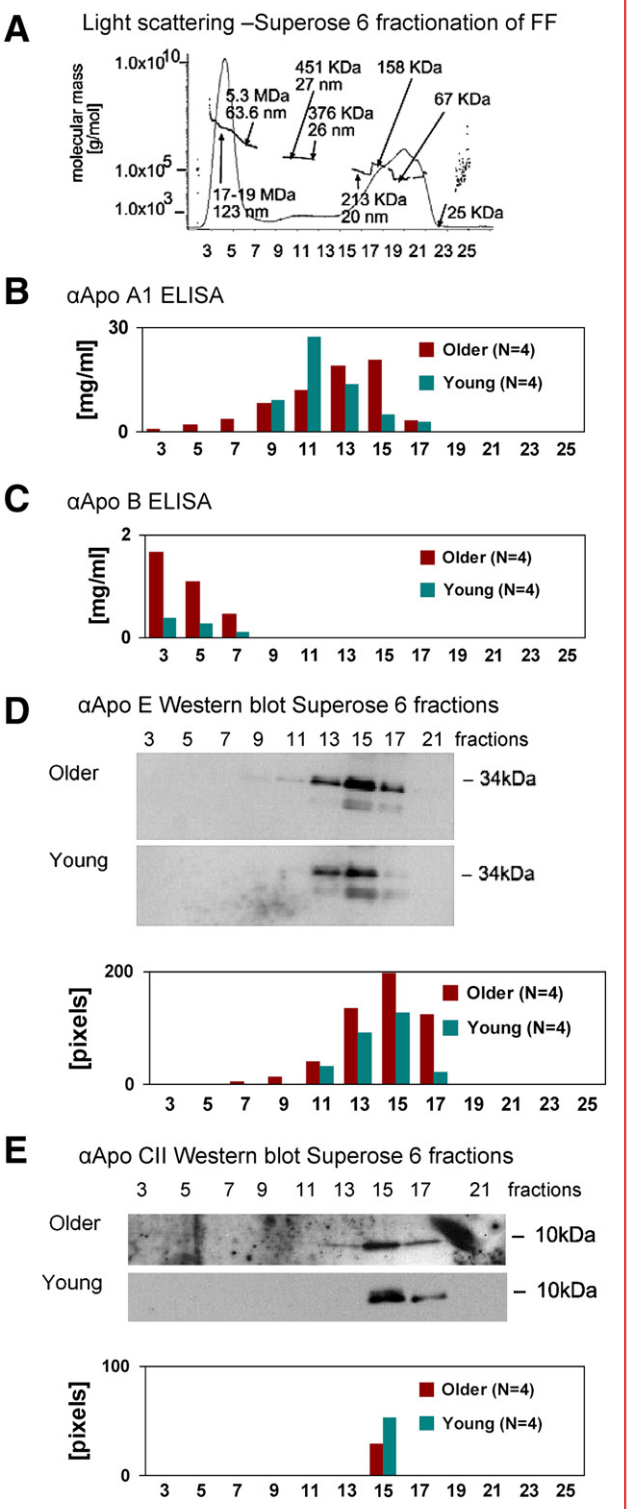

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FIGURE 2 Continued

Distribution of apolipoproteins in fractions generated by high-performance liquid chromatography (HPLC). Follicular fluid (FF) is subjected to gel filtration chromatography. The molecular mass of the constituents in the eluted fractions is determined by light scattering. The individual apolipoproteins in the elution profile are localized and measured by enzyme-linked immunosorbent assay (ELISA) with protein-specific antibodies. (**A**) Molar mass and size distribution plot for the eluted FF fractions as determined by light scattering. The solid line represents the trace from the light-scattering detector, and the broken line is the weighted average molecular weight in the fractions from the Superose 6 column, labeled on the logarithmic y-axis. (**B**) Apo A1 content in the eluted fractions is determined by ELISA. The individual bars represent the amount of Apo A1 determined by using a standard curve with recombinant Apo A1 protein (mg/mL). (**C**) Apo B content in the eluted fractions is determined by ELISA. (**D**) Apo E content in the eluted fractions is determined by Western blot with human Apo E–specific antibody. (**E**) Apo CII content in the eluted fractions is determined by Western blot with human Apo CII–specific antibody. The relative amount of Apo E and Apo CII in the fractions is shown in the histogram below the corresponding panels.

women for both VLDL (P=.008) and LDL (P=.008). Thus, FF contains not only HDL but also LDL and IDL, which differ in apolipoprotein composition from the corresponding blood plasma cholesterol particles. The follicular LDL and VLDL revealed statistically significant age-related differences in Apo E content.

Associations among Apolipoproteins and Apolipoprotein Particle Content, Aging, and Number of Retrieved Mature Oocytes

Because clinical outcomes of IVF are highly dependent upon age, we sought to identify a difference in variables related to oocyte development, including the total number of retrieved mature oocytes and the individual follicular apolipoprotein and apolipoprotein particle content. Cycle outcomes are shown in Table 1. There was no statistically significant difference between groups with respect to cycle number or BMI. As expected, the younger women had better fertility potential and a higher pregnancy rate (defined by a gestational sac seen on ultrasound) albeit not statistically significant, than the older women. The older women received higher doses of total gonadotropins (P<.001) and had a higher mean cycle day 3 FSH level (P=.01). The mean number of retrieved mature oocytes per woman was higher in the younger compared with older women (P=.03). Although the number of women in our study was not sufficient to determine statistically significant correlations among apolipoproteins and number of mature oocytes or other clinical outcome measures, the reported

FIGURE 3

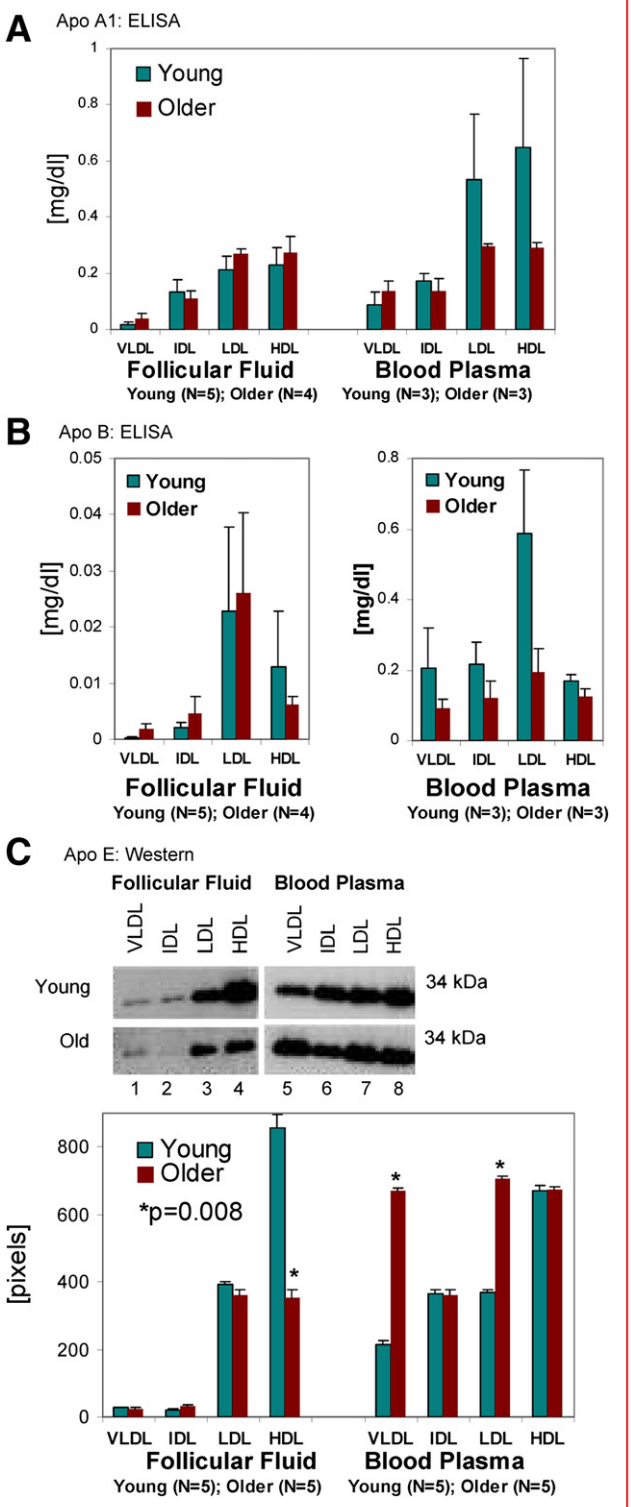

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FIGURE 3 Continued

High-density lipoproteins (HDL), intermediate-low-density lipoproteins (LDL), very-low-density lipoproteins (VLDL), and intermediate-low-density lipoproteins (IDL) presence and composition in follicular fluid (FF) and blood plasma. The FF and corresponding blood plasma samples were individually subjected to isopycnic ultracentrifugation to isolate HDL, LDL, VLDL, and IDL. (**A**) Apo A1 content in FF and blood plasma isopycnic ultracentrifugation fractions. (**B**) Apo B content measured by enzyme-linked immunosorbent assay (ELISA). Each bar represents the Apo A1 and Apo B content in the isopycnic fractions of VLDL, IDL, LDL, and HDL. (**C**) The FF and blood plasma isopycnic ultracentrifugation fractions analyzed for Apo E content by Western blot with Apo E–specific antibody. Lanes 1 to 4 show the immunologic reaction of FF fractions with anti–Apo E antibody; lanes 5 to 8 show the immunologic reaction of blood plasma fractions.

variations suggest that these apolipoproteins may play an important role in the age-related decline of oocyte maturation and decline in fertility potential.

DISCUSSION

Our results suggest an association between individual follicular apolipoprotein content and the decline in fertility with aging. The variation in FF apolipoprotein content may signify a marker for production of mature oocytes. We noted that apolipoproteins are present in diverse FF-specific cholesterol lipoprotein particles, including not only HDL as previously proposed (19, 20) but also LDL and some IDL. Exactly how the difference in the individual lipoproteins content translates to a lower number of mature oocytes is unknown. Their complex distribution status may direct follicular-specific functionality. Apo A1 may not only serve as a carrier for steroid precursors but also display antioxidant effects protecting the oocyte from toxic injury, thereby improving fertilization potential. Our recent data (unpublished) show that the aging process results in increased follicular presence of proinflammatory factors that are unhealthy for the developing oocyte. Apo A1 and HDL in younger women could have local anti-inflammatory effects similar to its systemic effects. As an LDL constituent, Apo A1 also could inhibit the formation of proinflammatory oxidized lipids, adding to its overall protective capacity of oocyte development. Although the mechanism of Apo A1–mediated oocyte maturation is not yet well understood, through the various plausible mechanisms mentioned, Apo A1 could serve as a candidate target and biomarker for oocyte maturation and fertility potential.

To our knowledge, ours is the first report of Apo B in human FF. The FF content of Apo B is markedly lower than in blood. In contrast to ApoA1, most of the follicular

TABLE 1

Cycle characteristics and outcomes of in vitro fertilization.

Clinical variable	Young women, mean (SD)	Older women, mean (SD)	*P* value
Age (y)	30.2 (3.5)	41.3 (1.3)	<.0001[a]
Cycle number	2.3 (1.3)	2.9 (1.7)	.38
BMI (kg/m^2)	25.6 (4.2)	24.4 (4.0)	.31
CD3 FSH (mIU/mL)	5.4 (1.8)	7.9 (2.1)	.01[a]
Total dose of gonadotropins (IU)	2148.9 (738.3)	3957.2 (1818.4)	.001[a]
No. of mature oocytes	11.6 (6.2)	7.6 (4.8)	.03[a]
No. of embryos transferred	2.0 (0.8)	3.2 (1.7)	.02[a]
Mature oocytes fertilized (%)	69.6 (21.2)	76.2 (17.1)	.31
Pregnancy rate (%)	59.1	37.5	.19

Note: BMI = body mass index; CD3 FSH = cycle day 3 follicle-stimulating hormone; SD = standard deviation.
[a] Statistically significant.

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Apo B is found in large LDL complexes (21). The function of Apo B in FF and its association with aging is presently unknown and remains to be established.

Total Apo E content increased in FF with age, and a substantial amount of Apo E is found in follicular LDL and HDL. In contrast to higher total Apo E amounts seen in older women, its content in HDL of young FF is at least two times greater than in older FF. The 35-kd Apo E glycoprotein, mostly produced in the liver but also in other tissues including the ovary, is thought to play a role in gonadal physiology (22). Part of the follicular Apo E is likely to be produced in the follicles by the granulosa cells (our unpublished results) and distributed in complexes that may have age and follicle-specific functions. The exact role of follicular Apo E in maturation of fertility-competent oocytes is presently unknown. One possibility is that Apo E–rich HDL may influence steroidogenesis by inhibiting follicular androgen synthesis (23), promoting healthy oocyte maturation. It is also possible that HDL containing Apo E facilitates the maturation of oocytes, more so than Apo E alone or in a complex with LDL.

We observed that follicular Apo E participates in complexes together with Apo CII. This 9-kd apolipoprotein contains a lipoprotein lipase cofactor and is known to activate several triacylglycerol lipases in blood. Clinically, Apo CII deficiency is similar to lipoprotein lipase deficiency. Our finding that part of follicular Apo CII is in complexes together with Apo E would suggest that Apo E could significantly interfere with follicular lipolysis by interacting with the lipoprotein lipase activator Apo CII. Our finding that younger patients have more Apo CII than older patients raises the possibility of an age-related difference in follicular lipoprotein metabolism and energy homeostasis. Furthermore, follicular IDL, LDL, and HDL may also contribute by keeping them separate, preventing binding and Apo CII inactivation.

Exactly how IDL, LDL, and HDL content may correlate with the quality of oocytes and age-related fertility outcome is not clear. In blood, the apolipoproteins have well-established functions in cholesterol and triglyceride trafficking. However, our preliminary data suggest that there are significantly fewer total cholesterol and triglycerides in FF than in blood plasma particles (J. Reindollar, unpublished). It is likely that follicular IDL, LDL, and HDL composition supports specific functions that differ from that of blood plasma.

Whether changes in specific apolipoprotein level and IDL, LDL, HDL distribution are entirely a function of the follicular response to aging (24, 25) remains to be explored. Women at an older reproductive age often require higher doses of gonadotropins during the IVF stimulation to produce a reasonable number of mature oocytes (26). The total dose of gonadotropins, however, could influence the level of individual apolipoprotein content. There are currently no published data evaluating the influence of high-dose gonadotropins on FF apolipoprotein content. The indication for treatment could also affect individual lipoprotein levels in blood and perhaps also in FF. Future experiments including a larger number of samples would significantly contribute to establishing precisely the role of apolipoproteins in fertility decline with aging.

The variations in apolipoprotein content and their complexes in FF represent a novel means for establishing potential biomarkers for fertility potential. Our observations provide evidence that follicular-specific apolipoproteins may be directly associated with age-related infertility. It is possible the apolipoprotein variation in FF may serve as

a marker for the decline in production of mature oocytes. Further studies are warranted to determine the exact mechanism of action of these proteins and the role of their complexes in human oocyte maturation and fertility potential.

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