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Authors

Bloom, Michael S
Kim, Keewan
Fujimoto, Victor Y
[et al.](#)

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Variability in the components of high density lipoprotein particles measured in human ovarian follicular fluid - A cross-sectional analysis

Michael S. Bloom, PhD^{a,b,*}, Keewan Kim, MPH^a, Victor Y. Fujimoto, MD^c, and Richard W. Browne, PhD^d

^aDepartment of Environmental Health Sciences, University at Albany, State University of New York, Rensselaer, NY, USA

^bDepartment of Epidemiology and Biostatistics, University at Albany, State University of New York, Rensselaer, NY, USA

^cDepartment of Obstetrics, Gynecology, and Reproductive Sciences, University of California at San Francisco, San Francisco, CA, USA

^dDepartment of Biotechnical and Clinical Laboratory Sciences, University at Buffalo, State University of New York, Buffalo, NY, USA

Abstract

Objective—Assess the variability of follicular fluid (FF) high-density lipoprotein (HDL) particle components.

Design—FF specimens were collected from two contralateral follicles on the day of oocyte retrieval and analyzed for HDL components. We characterized analytes by age, body mass index (BMI), race and smoking using a cross-sectional design. Biological variability was assessed using two-stage nested analysis of variance.

Setting—Reproductive health center.

Patients—One-hundred eighty in vitro fertilization (IVF) patients.

Interventions—None.

Main Outcome Measures—Nineteen HDL components including HDL-cholesterol and free (unesterified) and esterified forms, phospholipids, triglycerides, apolipoproteins A-1 and A-2, paraoxonase 1 (PON1) activities, and seven lipophilic vitamins and micronutrients.

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*Corresponding author: Michael S. Bloom, PhD, Department of Environmental Health Sciences, School of Public Health Rm. #157, One University Place, Rensselaer, NY 12144 USA, Phone: (518) 473-1821, Fax: (518) 402-0380, mbloom@albany.edu.

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Results—For some analytes, a majority of total measurement variability was attributed to sources between-follicles, suggesting an important role for the integrity of the blood-follicle-barrier and *in situ* remodeling of plasma derived constituents. For other analytes, variability was mostly attributed to sources between-women, likely indicative of plasma levels. Variability between-follicles decreased with increasing age, differed by BMI and smoking, and generally were lower for Asians and women with diminished ovarian reserve.

Conclusions—Substantial variability in FF HDL components exist between-follicles among women undergoing IVF, as well as between-women by age, BMI, race, smoking and by infertility diagnosis.

Keywords

Biological variability; follicular fluid (FF); high density lipoprotein (HDL); *in vitro* fertilization (IVF); infertility

Introduction

In the pre-ovulatory follicle, mammalian oocytes are surrounded by cumulus granulosa cells and bathed by follicular fluid (FF) containing various proteins, lipids, sugars, hormones and metabolites (1). The relative composition of FF plays a critical role in supporting oocyte development and competence (2). We previously identified FF high density lipoprotein (HDL) - cholesterol and apolipoprotein A-1 (ApoA-1) (3), as well as FF β -cryptoxanthin and γ -tocopherol (4) as predictors of embryo quality following *in vitro* fertilization (IVF). Investigators have characterized endogenous human FF constituents including hormones, proteins, reactive oxygen species, amino acids and sugars, as well as dissolved O₂ (5). Several groups described concentrations of HDL-particle associated lipids, proteins, micronutrients and enzyme activities in the human ovarian follicle (3, 4, 6–10). Interest in the use of FF as a source of biomarkers predictive of IVF outcomes has gained tremendous momentum, although with limited success to date (5, 11).

HDL is the predominant class of lipoprotein found in human ovarian FF, a result of size restrictions introduced by the follicular basal lamina (12). Other lipoproteins and apolipoproteins, if present, are unmeasurable by routine assay (13); low density and very low density lipoprotein particles are not found in FF. Despite the unique lipoprotein composition of FF, sources of FF HDL variability have not been described, limiting its utility as a biomarker of the follicular environment. Commonly employed study designs in which pooled FF from multiple follicles were analyzed in relation to derived oocyte cohorts would be invalid for biomarkers that have significant variability between-follicles. To address this important data gap, we analyzed FF specimens collected from single follicles for a large panel of HDL-particle components. Our objective was to describe the biologic variability of FF HDL-particle components in women using a cross-sectional study design.

Methods

Sample selection

The study population was comprised of women referred to the University of California at San Francisco (UCSF) Center for Reproductive Health for infertility treatment. Between April 10th, 2010 and June 28th, 2011, a convenience sample of 180 women undergoing IVF treatment with fresh, non-donor oocytes was recruited by a research assistant. The participation rate was 97.8% (n=4 refusals) and there were no exclusion criteria. All participants received a comprehensive infertility evaluation and completed a questionnaire to ascertain health-related behaviors including smoking. Height and weight were measured by a standard procedure, and body mass index (BMI) was calculated as weight divided by height squared. Pre-cycle informed consent was obtained and the study protocol was approved by the UCSF Committee on Human Research.

Clinical protocol and specimen collection

Participants underwent controlled gonadotropin-induced ovarian stimulation (COS) according to standard clinic protocols. Endometrial development and follicle maturation were monitored using transvaginal ultrasound and serum estradiol (E₂). When a sufficient number of follicles > 7 mm diameter developed human chorionic gonadotropin (hCG) was administered subcutaneously. Oocytes were retrieved using transvaginal needle (18 gauge) aspiration 36 hours later. Participants were instructed to fast for at least eight hours to facilitate conscious sedation during the procedure. Contralateral follicles were collected from most women (n=171). The first follicle from each ovary was aspirated using a clean, never-used needle or a fully washed needle for a second collection and individually evacuated into an empty 10 mL tube and processed. The largest follicle was aspirated and we only sampled follicles > 7 mm diameter. Follicles were not flushed in order to preserve the native concentrations of FF analytes. Ipsilateral follicles were sampled from six women and three women had only a single sampled follicle. After removal of the oocyte, each individual 3.5–5.0 mL aspirate was centrifuged to pellet the residual granulosa cells. The FF supernatant was aspirated and split into aliquots (0.6 mL), and then frozen at –80°C. Any samples showing evidence of red blood cells either before or after centrifugation were not analyzed (14). Our protocol ensured that each specimen came from a single follicle and reduced the possibility for blood contamination. Two aliquots from each follicle were shipped to the University of Buffalo (Buffalo, NY), on dry ice via overnight service.

Analytic methods

ApoA-1, apolipoprotein A-2 (ApoA-2) and paraoxonase (*PONI*) activities were performed directly on whole FF. For the remaining analytes, FF-HDL fractions were prepared by selective precipitation to remove any trace amounts of apolipoprotein B containing low density lipoproteins as previously described (3). Briefly, one volume of precipitating reagent (1.6 mmol/L phosphotungstic acid, 13.6 mmol/L magnesium chloride) was added to five volumes of FF. After 15 minutes incubation, the sample was centrifuged at 16,000 × g in a microcentrifuge and the total HDL containing supernatant was collected.

FF HDL-particle lipids including cholesterol, phospholipids and triglycerides were measured using diagnostic reagent kits from Sekisui Diagnostics Inc. (Lexington, MA), adapted to the Cobas Fara II automated chemistry analyzer (Hoffmann-La Roche and Co., Switzerland) and reported as mg/dL. Assays were controlled using Sekisui's DC-TROL, 3-level quality control (QC) material. The interassay coefficients of variation (CV) were 2.6% for cholesterol, 3.6% for phospholipids and 4.8% for triglycerides using the low level QC, which was most similar to FF levels. ApoA-1 and ApoA-2 levels were analyzed by immunoturbidometric methods using diagnostic kits from Kamiya Biomedical Co. (Tukwila, WA) also on the Cobas Fara II. Assays were controlled using 2-level, lyophilized, apolipoprotein QC material. The interassay CVs were 4.6% for ApoA-1 and 4.6% for ApoA-2 using the low level QC.

Free (unesterified) cholesterol and cholesteryl esters (including cholesteryl palmitate, oleate, linoleate and arachidonate) were measured by high performance liquid chromatography (HPLC) according to modification of a previously described method (15). Cholesterols were detected by their UV absorbance at 208 nm and quantified in comparison to pure standards using cholesteryl heptadecanoate as an internal standard. The HPLC assay was controlled using Sekisui's DC-TROL, 3-level QC material. Inter-assay CVs were 1.2% for free cholesterol and <4.4% for each of the individual cholesteryl esters. HDL-particle component micronutrients including vitamin A (retinol), vitamin E (α and γ tocopherols) and carotenoids (β -carotene, β -cryptoxanthin, lycopene and lutein/zeaxanthin) were measured simultaneously using HPLC according to a previously described method, and reported as $\mu\text{g/mL}$ (16). Lutein and zeaxanthin were quantified as a single co-eluting peak. The HPLC assay was validated by continuous participation in the National Institutes of Standards and Technology (NIST) micronutrients measurement quality assurance program proficiency testing and controlled using NIST standard reference material (SRM) 968c. The interassay CV of retinol was 2.4%, α -tocopherol 3.6%, γ -tocopherol 6.8%, β -carotene 6.7%, β -cryptoxanthin 7.2%, lycopene 3.5% and lutein/zeaxanthin 5.6%.

Arylesterase and paraoxonase activities were determined precisely as previously described (17). An in-house human serum pool frozen at -70°C was used for quality control. The CVs of *PONI* arylesterase and paraoxonase activity assays were 0.6% and 1.4% respectively.

Statistical methods

Distributions of demographic and clinical factors and analytes were characterized for 342 contralateral follicle aspirates collected from 171 women (Supplemental Figure 1). Automated chemistry analyses were determined in duplicate and included HDL-cholesterol, phospholipids, triglycerides, ApoA-1, ApoA-2 and PON1 paraoxonase and arylesterase activities; these we defined as 'Group I.' Because of limited sample volumes, a single determination was made for analytes measured by HPLC, including free cholesterol, and cholesteryl palmitate, oleate, linoleate and arachidonate, retinol, β -carotene, β -cryptoxanthin, α -tocopherol, γ -tocopherol, lutein/zeaxanthin and lycopene; these we defined as 'Group II.' We used a natural log transformation to normalize analyte distributions and to stabilize variances. Values were compared by demographic factors related to IVF outcomes, including age (<35 vs. ≥ 35 years) (18), BMI (<25 kg/m^2 vs. ≥ 25 and <30 kg/m^2 vs. ≥ 30

kg/m²) (19, 20), race (non-Asian vs. Asian) (21) and cigarette smoking (never vs. ever) (22), and by clinical factors including infertility diagnosis (23) and COS protocol using one-way analysis of variance (ANOVA), followed by Student t-tests if statistically significant to identify between group differences.

Using two-stage nested ANOVA we characterized sources of variability due to factors between-women and between-follicles, and due to analytic factors among women with 'balanced' data (i.e. no missing values). We also calculated relative contributions to total measurement variability, and determined CVs and intraclass-correlation coefficients (ICC). We assessed differences between the contribution of between-follicles sources for demographic and clinical groups by examining confidence intervals (24). A more detailed description of the statistical analysis is provided as a supplemental methods file. SAS v.9.3 (SAS Institute, Cary, NC) was used for analysis and statistical significance was defined as $P < 0.05$ for a two-tailed test.

Results

Distributions of demographic factors and clinical factors

Distributions of demographic and clinical factors are presented in Table 1. A majority of women were non-Asian (71%), primarily white, with few Hispanics (5.8%). Primary infertility diagnosis was grouped *a priori* as 'male factor' (MF), 'unexplained' (including $n=1$ recurrent loss), 'non-diminished ovarian reserve female infertility' (non-DOR; $n=10$ endometriosis, $n=10$ tubal, $n=7$ polycystic ovary syndrome and $n=3$ anovulation) or 'diminished ovarian reserve female infertility' (DOR; $n=27$); $n=4$ underwent preimplantation genetic diagnosis. The COS protocols were grouped as Lupron down-regulated (LDR; $n=94$ long luteal, $n=20$ demi-halt, and $n=1$ very low dose LDR), antagonist ($n=26$ E₂ priming antagonist and $n=16$ oral contraceptive pill antagonist), or flare ($n=2$ Clomid flare, $n=11$ microdose flare, and $n=1$ Clomid/follicle-stimulating hormone).

Measurement variability sources, FF HDL-particle components

Geometric means and standard deviations (SD) for analytes, and proportions of total measurement variability attributed to different sources are characterized in Table 2. Analytic factors ($\% \sigma^2_A$) made nominal contributions to the variability of Group I analytes. The single determination prevented the calculation of analytic variability for Group II analytes. Consequently, estimates of relative variability between-follicles ($\% \sigma^2_F$) for Group II analytes include the unspecified analytic component. All estimates of variability between-follicles differed significantly from zero. Relative variability contributed by sources between-women ($\% \sigma^2_B$) was generally greater than for sources between-follicles, although the pattern was reversed for ApoA-2 and cholesteryl palmitate. Ratios of variability ($\% \sigma^2_F : \% \sigma^2_B$) were 0.5 for HDL-cholesterol, phospholipids, apolipoproteins, free cholesterol and for most cholesteryl esters. In contrast, triglycerides, arylesterase and paraoxonase and most micronutrients had ratios of variability well below 0.5. ICCs were 0.80 for triglycerides, arylesterase and paraoxonase, as well as for all micronutrients with the exceptions of retinol and α -tocopherol (Table 2). Supplemental Figure 2 provides 95% CIs around the ICCs.

Distribution of FF analytes by demographic and clinical factors

No differences (δ) in FF analytes were detected by age. Compared to women with BMI <25 kg/m² those with BMI \geq 30 kg/m² had lower geometric mean levels of phospholipids (δ =12.97 mg/dL; P=0.01), free cholesterol (δ =0.35 mg/dL; P=0.003), cholesteryl oleate (δ =1.54 mg/dL; P=0.002), cholesteryl linoleate (δ =1.91 mg/dL, P=0.02), β -carotene (δ =0.03 mg/mL; P=0.003), β -cryptoxanthin (δ =0.02 mg/mL; P=0.0003), α -tocopherol (δ =0.46 mg/mL; P=0.01), lutein/zeaxanthin (δ =0.03 mg/mL; P=0.001), and lycopene (δ =0.02 mg/L; P=0.03). In contrast, triglycerides (δ =3.86 mg/dL; P=0.004) and γ -tocopherol (δ =0.09 mg/mL; P=0.01) were higher among women \geq 30 kg/m² than for <25 kg/m². Paraoxonase was higher in Asians (δ =28.91 IU/L; P<.0001) whereas cholesteryl arachidonate was lower (δ =0.60 mg/dL; P=0.04), and the ever-smokers had higher HDL cholesterol than the never-smokers (δ =4.19 mg/dL; P=0.05). No significant differences were identified by clinical factors. Compared to DOR, unexplained infertility had higher ApoA-2 (δ =3.39 mg/dL; P=0.05) and cholesteryl oleate (δ =1.12 mg/dL; P=0.02). Compared to women receiving an antagonist COS protocol, LDR had higher HDL-cholesterol (δ =2.77 mg/dL; P=0.04) and cholesteryl oleate (δ =0.73 mg/dL; P=0.02).

Measurement variability sources, FF HDL-particle components: Demographic factors

We assessed proportions of total measurement variability attributable to various sources by demographic factors (Table 3). The relative analytic variability was closely similar across strata, indicating consistent and modest contributions to the total variability. Between-follicles variability was significantly different from zero for all demographic subgroups. The n for each of the groups varied slightly due to missing demographic data and limited FF (Supplemental Table 1 provides the precise n for each variable).

Compared to women \geq 35 years of age, those <35 years had significantly greater relative between-follicle variability for triglycerides, β -carotene, β -cryptoxanthin and γ -tocopherol. Compared to other women, women with BMI \geq 30 kg/m² had significantly higher relative between-follicle variability for triglycerides, β -cryptoxanthin and lutein/zeaxanthin, whereas the relative variability between-follicles were highest among women with 25 \leq BMI <30 kg/m² for cholesteryl palmitate and retinol. By race, Asians had higher relative variability between-follicles than non-Asians for arylesterase and paraoxonase, but lower for retinol, β -carotene, β -cryptoxanthin, α -tocopherol and lycopene. Finally, never-smokers, demonstrated significantly larger relative variability between-follicles than ever-smokers for ApoA-2, free cholesterol and cholesteryl arachidonate, with a reversed pattern for ApoA-2 and γ -tocopherol.

Measurement variability sources, FF HDL-particle components: Clinical factors

We also assessed proportions of total measurement variability attributable to various sources by clinical factors (Table 4). The relative analytic variability was again similar across strata, indicating that laboratory variability sources did not differ by group. The contribution of sources between-follicles differed significantly from zero (P<0.05) for all clinical subgroups. The n for each of the groups varied slightly due to issues related to limited FF (Supplemental Table 2 provides the precise n for each variable).

Women with DOR had significantly smaller relative between-follicles variability than other participants for HDL-cholesterol, triglycerides, arylesterase and paraoxonase. In addition, the relative variability of triglycerides between-follicles was significantly smaller among women with DOR compared to women with MF and larger for cholesteryl palmitate. Between follicles variability was significantly lower among women with unexplained infertility compared to other diagnoses for β -cryptoxanthin and lutein/zeaxanthin, compared to women with non-DOR for α -tocopherol, and compared to women with MF and DOR for ApoA-2. Women with non-DOR also had the highest relative between-follicles γ -tocopherol variability.

Compared to other protocols, relative variability between-follicles were higher for paraoxonase and β -cryptoxanthin among LDR and ApoA-1 was lower for flare. LDR also had higher relative variability between-follicles than flare for arylesterase and lycopene, and for ApoA-2 and γ -tocopherol compared to antagonist. The relative variability between-follicles for free cholesterol was significantly smaller among LDR than antagonist, yet the relative between-follicles β -carotene variability was smallest for antagonist. Flare demonstrated the lowest relative between-follicles HDL-cholesterol variability.

Discussion

In this study we analyzed components of HDL-particles in FF specimens collected from women undergoing IVF, and estimated the components of biological variability in order to better understand these biomarkers. We detected substantial variation in sources contributing to the total measurement variability of analytes. For HDL-cholesterol, free cholesterol, phospholipids and cholesteryl esters, sources between-follicles were of greatest relevance. For triglycerides, *PON1* enzymes and micronutrients, sources of variability between-women made proportionately larger contributions to the total. The relative contribution of between-follicles sources decreased with age for triglycerides and micronutrients, and was also associated with BMI. The relative between-follicles variability of *PON1* enzymes and micronutrients were associated with race, and both micronutrients γ -tocopherol and ApoA-2 varied by cigarette-smoking. Infertility diagnosis was also associated with the between-follicles variability of FF analytes, with DOR tending towards lower variability than other diagnoses, and the COS protocol appeared important for many biomarkers including *PON1* enzymes, apolipoproteins, free cholesterol and most micronutrients. Among the apolipoproteins, sources of variability between-women made a greater impact for ApoA-1, yet the total variability for ApoA-2 was dominated by sources between-follicles.

Concentrations of many FF analytes in our study decreased with increasing BMI, including β -cryptoxanthin, β -carotene and α -tocopherol, although increases were indicated for triglycerides and γ -tocopherol. In our earlier work, higher FF γ -tocopherol adjusted β -cryptoxanthin was protective against embryo fragmentation (4), although another group reported no difference in FF carotenoids, including β -cryptoxanthin, for pregnant and not-pregnant women following IVF (8). Higher FF γ -tocopherol might correspond to generalized nutrient intake as the γ isomer is commonly encountered in U.S. diets (25). Similar associations between HDL-particle associated analytes and body mass have been widely reported for human plasma (26, 27). Consistent with our observation, higher FF

triglycerides were reported in association with increased BMI among South Australian ($r=0.45$; $P=0.0003$) (28) and Belgian ($P=0.03$) (29) IVF patients; yet, there were no associations for FF cholesterol or ApoA-1 in the latter study. Lower average plasma β -carotene (30) and α -tocopherol (31) levels were previously reported for obese girls and women, respectively. Prior studies reported reduced plasma paraoxonase in overweight or obese women (32, 33), although we did not detect a difference in FF.

PON1 paraoxonase was higher in Asians than in non-Asians, consistent with previous work (34). Although they are promiscuous substrates, paraoxon (paraoxonase) and phenylacetate (arylesterase) are the most widely used estimates of Paraoxonase 1 activity (35) which is believed to account in large part for the antioxidant activities of HDL (36, 37). We previously reported a positive association for FF arylesterase with embryo cleavage rate following IVF (3), although a difference was not detected here. This disparity in *PON1* paraoxonase activity may in part account for the better performance of Asian women during IVF up to the point of implantation (38, 39). We did not detect previously reported differences in lipophilic micronutrients by cigarette smoking (40, 41), although FF HDL-cholesterol was significantly increased among smokers. While an inverse association between cigarette smoking and circulating HDL-cholesterol has long been recognized (42), we found no prior reports characterizing the association in FF. This difference might reflect a unique facet of follicular physiology, but we conducted many statistical tests and so it may be a chance finding.

FF ApoA-2 and cholesteryl oleate differed by diagnosis; there were decreased concentrations in women with DOR compared to those whose diagnosis was unexplained. We detected no differences in HDL lipoprotein particle components by diagnosis or clinical IVF protocols in our study. Analogous results were described for a recent study reporting similar FF HDL cholesterol and protein levels between women with DOR and women with normal ovarian reserve (43). We previously reported an inverse association between FF ApoA-1 adjusted HDL-cholesterol and embryo fragmentation (3). However, a more recent report described lower FF HDL levels for oocytes that developed into early-stage embryos compared to those that did not (10). Earlier studies reported longitudinal increases in plasma HDL-cholesterol and ApoA-1 during a standard LDR IVF protocol (44), and hypertriglyceridemia following treatment with clomiphene citrate (45). We did not collect baseline specimens and thus we were unable to assess changes over time. We were also unable to compare levels to an untreated reference as our study was conducted within the context of standard clinical protocols.

Variability of FF analytes attributed to sources between-women generally exceeded that attributed to sources between-follicles in our study. For between-follicles variability, we suggest that follicular factors, including the microfollicular vasculature, the permeability/integrity of the blood-follicle barrier and/or intra-follicular metabolism, are the driving factors. FF is, to a great extent, plasma derived as most FF proteins are plasma proteins (13). There is also a generally high degree of correlation between blood plasma and FF HDL component levels (4). With no direct blood supply, the growing follicle is nourished via diffusion from a surrounding capillary network. Studies using Doppler ultrasonography described wide variation in the extent of the microvasculature for adjacent follicles, which

was correlated to the dissolved O₂ content and was related to embryo quality (46, 47). Levels of FF vascular endothelial growth factor (VEGF), a mediator of vascular permeability (48), were correlated to FF O₂ levels (46) suggesting the importance of the blood-follicle-barrier and *in situ* metabolism.

Paraoxonase 1 activity is determined to a great extent by the *PONI* Q192R polymorphism which accounts for 95% of the biological variability observed in human plasma for this biomarker (17). We observed a similar relative contribution from sources between-women to the total variance in the samples studied. This indicates that the follicle environment does not substantially alter this biomarker. Similar observations can be made for biomarkers strongly related to dietary behaviors, such as β -carotene, β -cryptoxanthin and γ -tocopherol. Accordingly, we suggest that these biomarkers would be less reflective of the follicular environment and FF measurement would not improve upon a more routine blood analysis. Conversely, we propose that biomarkers with high ratios of variability, such as ApoA-2, will have greater follicular influence and may be useful indicators of follicular metabolism, integrity, maturity and quality.

The relative contributions of variability sources between-follicles varied by age, BMI, race and smoking; women <35 years tended to have greater relative between-follicles variability than women >35 years, although patterns for BMI, race and smoking were unclear. Increased age (49), BMI (19) and smoking (22) are recognized as negative predictors of female fertility and of IVF success. In fact, variability sources between-follicles made greater relative contributions to HDL-cholesterol, phospholipids, and ApoA-1 among younger women, although the differences were not significant, and to triglycerides, for which the difference was significant. It is tempting to speculate, based on these data, that the above factors influence perivascular development and consequently metabolic potential in the follicle, pre-ordaining the destiny of the oocyte within.

We evaluated CVs and ICCs for FF analytes to assess their potential use as biomarkers in epidemiologic studies (50). The CV describes the precision with which the geometric mean value was measured. CVs were below 10%, a frequently employed clinical threshold for reliability (51), for all Group I analytes with the exception of triglycerides. Yet, CVs exceeded 10% for all Group II analytes. Given similar variance estimates, these patterns likely reflect the lower average concentrations for Group II relative to the Group I, and thus the increased difficulty in reliable mean value estimation. The ICC describes the proportion of observed variability associated with 'true' differences between-women. Values <0.80 introduce considerable exposure measurement misclassification into a study, and this value is a frequently employed threshold to determine adequacy for use in epidemiologic studies (52). ICC point estimates exceeded 0.80 for triglycerides, paraoxonase, β -carotene, β -cryptoxanthin, γ -tocopherol, lutein/zeaxanthin and lycopene, although 95% CIs mostly overlap 0.80, indicating limitations as biomarkers.

Several important factors limit the results of our study. Changes in the levels of FF constituents are well-recognized in association with mammalian follicle growth, including cholesterol, triglycerides and total proteins (53). Data from ovine follicles suggest changes in HDL-particle components with increasing follicle size (54). Although we were unable to

adjust for follicle size, we sampled the largest follicle in each ovary with diameter ≥ 17 mm and so we anticipate the impact was modest. In addition, with no follicles sampled from ipsilateral ovaries we were unable to isolate variability due to sources between-ovaries. The expense and sample volume requirements of HPLC also precluded dual determinations for Group II analytes, and thus we were unable to isolate the impact of analytic factors. However, based on analytical performance of the HPLC assays and previous estimates of analytical variability (55), we are confident it was similarly small for Group II analytes. Our protocol was intended to minimize blood contamination in FF specimens and ensured collection from single follicles. Still, unrecognized blood contamination is a possibility. Because of small sample sizes we collapsed some groups, which might have obscured their differences (e.g., BMI <18.5 kg/m² included with BMI <25 kg/m², BMI >40 kg/m² included with BMI ≥ 30 kg/m²) and we did not adjust for potential confounding in comparisons. However, our intent was to identify influential factors for FF HDL-particle variability and so we *a priori* decided to focus on unadjusted associations.

In conclusion, we suspect that substantial between-follicles variability reflects metabolic activity and particle remodeling within the ovarian follicle. Between-women variability is more likely to be a function of unmodified plasma lipid concentrations, analogous to prior reports for FF proteins (13). Whereas FF HDL-particle biomarkers determined primarily by plasma concentrations sources might be well-suited to composite, or ‘oocyte cohort’ study designs, those which undergoing substantial within-follicle modification will require a ‘one-follicle-one-oocyte’ design for study. Women undergoing IVF comprise a highly selected group (56), with financial resources sufficient to facilitate treatment (57), and so these study results should be generalized to other populations with caution. Still these results have important implications in further elucidating the role of follicular function in the etiology of female infertility and clinical IVF outcomes. The variability observed is an important consideration for the design of future studies on the impact of follicular dynamics on IVF outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Edwards RG. Follicular fluid. *J Reprod Fertil.* 1974; 37:189–219. [PubMed: 4274030]
2. Hennet ML, Combelles CMH. The antral follicle: A microenvironment for oocyte differentiation. *Int J Dev Biol.* 2012; 56:819–31. [PubMed: 23417404]
3. Browne RW, Shelly WB, Bloom MS, Ocque AJ, Sandler JR, Huddleston HG, et al. Distributions of high-density lipoprotein particle components in human follicular fluid and sera and their associations with embryo morphology parameters during IVF. *Hum Reprod.* 2008; 23:1884–94. [PubMed: 18487218]

4. Browne RW, Bloom MS, Shelly WB, Ocque AJ, Huddleston HG, Fujimoto VY. Follicular fluid high density lipoprotein-associated micronutrient levels are associated with embryo fragmentation during IVF. *J Assist Reprod Genet.* 2009; 26:557–60. [PubMed: 19921421]
5. Revelli A, Piane LD, Casano S, Molinari E, Massobrio M, Rinaudo P. Follicular fluid content and oocyte quality: From single biochemical markers to metabolomics. *Reprod Biol Endocrinol.* 2009; 7:40. [PubMed: 19413899]
6. Fujimoto VY, Bloom MS, Huddleston HG, Shelley WB, Ocque AJ, Browne RW. Correlations of follicular fluid oxidative stress biomarkers and enzyme activities with embryo morphology parameters during in vitro fertilization. *Fertil Steril.* 2011; 96:1357–61. [PubMed: 22014879]
7. Carbone MC, Tatone C, Delle Monache S, Marci R, Caserta D, Colonna R, et al. Antioxidant enzymatic defences in human follicular fluid: Characterization and age-dependent changes. *Mol Hum Reprod.* 2003; 9:639–43. [PubMed: 14561807]
8. Schweigert FJ, Steinhagen B, Raila J, Siemann A, Peet D, Buscher U. Concentrations of carotenoids, retinol and α -tocopherol in plasma and follicular fluid of women undergoing IVF. *Hum Reprod.* 2003; 18:1259–64. [PubMed: 12773456]
9. Jaspard B, Collet X, Barbaras R, Manent J, Vieu C, Parinaud J, et al. Biochemical characterization of pre- β 1 high-density lipoprotein from human ovarian follicular fluid: Evidence for the presence of a lipid core. *Biochemistry (Mosc).* 1996; 35:1352–7.
10. Wallace M, Cottell E, Gibney MJ, McAuliffe FM, Wingfield M, Brennan L. An investigation into the relationship between the metabolic profile of follicular fluid, oocyte developmental potential, and implantation outcome. *Fertil Steril.* 2012; 97:1078–84. e8. [PubMed: 22365382]
11. Baskind NE, McRae C, Sharma V, Fisher J. Understanding subfertility at a molecular level in the female through the application of nuclear magnetic resonance (NMR) spectroscopy. *Hum Reprod Update.* 2011; 17:228–41. [PubMed: 20801939]
12. Le Goff D. Follicular fluid lipoproteins in the mare: Evaluation of HDL transfer from plasma to follicular fluid. *Biochim Biophys Acta.* 1994; 1210:226–32. [PubMed: 8280774]
13. Angelucci S, Ciavardelli D, Di Giuseppe F, Eleuterio E, Sulpizio M, Tiboni GM, et al. Proteome analysis of human follicular fluid. *Biochimica et Biophysica Acta - Proteins and Proteomics.* 2006; 1764:1775–85.
14. Levay PF, Huyser C, Fourie FLR, Rossouw DJ. The detection of blood contamination in human follicular fluid. *J Assist Reprod Genet.* 1997; 14:212–7. [PubMed: 9130069]
15. Gay CA, Stocker R. Simultaneous determination of coenzyme Q10, cholesterol, and major cholesterylesters in human blood plasma. *Methods Enzymol.* 2004; 378:162–9. [PubMed: 15038967]
16. Browne RW, Armstrong D. Simultaneous determination of serum retinol, tocopherols, and carotenoids by HPLC. *Methods Mol Biol.* 1998; 108:269–75. [PubMed: 9921536]
17. Browne RW, Koury ST, Marion S, Wilding G, Muti P, Trevisan M. Accuracy and biological variation of human serum paraoxonase 1 activity and polymorphism (Q192R) by kinetic enzyme assay. *Clin Chem.* 2007; 53:310–7. [PubMed: 17185369]
18. Sharma V, Allgar V, Rajkhowa M. Factors influencing the cumulative conception rate and discontinuation of in vitro fertilization treatment for infertility. *Fertil Steril.* 2002; 78:40–6. [PubMed: 12095488]
19. Rittenberg V, Seshadri S, Sunkara SK, Sobaleva S, Oteng-Ntim E, El-Toukhy T. Effect of body mass index on IVF treatment outcome: An updated systematic review and meta-analysis. *Reprod Biomed Online.* 2011; 23:421–39. [PubMed: 21885344]
20. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults - The evidence report. *Obes Res.* 1998; 6:XI-209S.
21. Fujimoto VY, Luke B, Brown MB, Jain T, Armstrong A, Grainger DA, et al. Racial and ethnic disparities in assisted reproductive technology outcomes in the United States. *Fertil Steril.* 2010; 93:382–90. [PubMed: 19081561]
22. Neal MS, Foster WG, Younglai EV. The detrimental effect of smoking on female fertility and IVF success. *Curr Womens Health Rev.* 2008; 4:16–24.

23. van Loendersloot LL, van Wely M, Limpens J, Bossuyt PMM, Repping S, van der Veen F. Predictive factors in in vitro fertilization (IVF): A systematic review and meta-analysis. *Hum Reprod Update*. 2010; 16:577–89. [PubMed: 20581128]
24. Julious SA. Using confidence intervals around individual means to assess statistical significance between two means. *Pharm Stat*. 2004; 3:217–22.
25. Wagner KH, Kamal-Eldin A, Elmadfa I. Gamma-tocopherol - An underestimated vitamin? *Ann Nutr Metab*. 2004; 48:169–88. [PubMed: 15256801]
26. Glueck CJ, Taylor HL, Jacobs D, Morrison JA, Beaglehole R, Williams OD. Plasma high-density lipoprotein cholesterol: Association with measurements of body mass. The Lipid Research Clinics Program Prevalence Study. *Circulation*. 1980; 62:IV-62–9.
27. Lamou-Fava S, Barnett JB, Woods MN, McCormack C, McNamara JR, Schaefer EJ, et al. Differences in serum sex hormone and plasma lipid levels in Caucasian and African-American premenopausal women. *J Clin Endocrinol Metab*. 2005; 90:4516–20. [PubMed: 15886254]
28. Robker RL, Akison LK, Bennett BD, Thrupp PN, Chura LR, Russell DL, et al. Obese women exhibit differences in ovarian metabolites, hormones, and gene expression compared with moderate-weight women. *J Clin Endocrinol Metab*. 2009; 94:1533–40. [PubMed: 19223519]
29. Valckx SDM, De Pauw I, De Neubourg D, Inion I, Berth M, Franssen E, et al. BMI-related metabolic composition of the follicular fluid of women undergoing assisted reproductive treatment and the consequences for oocyte and embryo quality. *Hum Reprod*. 2012; 27:3531–9. [PubMed: 23019302]
30. Kuno T, Hozumi M, Morinobu T, Murata T, Mingci Z, Tamai H. Antioxidant vitamin levels in plasma and low density lipoprotein of obese girls. *Free Radic Res*. 1998; 28:81–6. [PubMed: 9554835]
31. Myara I, Alamowitch C, Michel O, Heudes D, Bariety J, Guy-Grand B, et al. Lipoprotein oxidation and plasma vitamin E in nondiabetic normotensive obese patients. *Obes Res*. 2003; 11:112–20. [PubMed: 12529493]
32. Ferretti G, Bacchetti T, Moroni C, Savino S, Liuzzi A, Balzola F, et al. Paraoxonase activity in high-density lipoproteins: A comparison between healthy and obese females. *J Clin Endocrinol Metab*. 2005; 90:1728–33. [PubMed: 15613429]
33. Ferretti G, Bacchetti T, Masciangelo S, Bicchiega V. HDL-paraoxonase and membrane lipid peroxidation: A comparison between healthy and obese subjects. *Obesity*. 2010; 18:1079–84. [PubMed: 19834469]
34. Ali SM, Chia SE. Interethnic variability of plasma paraoxonase (PON1) activity towards organophosphates and PON1 polymorphisms among Asian populations - A short review. *Ind Health*. 2008; 46:309–17. [PubMed: 18716378]
35. Gan KN, Smolen A, Eckerson HW, La Du BN. Purification of human serum paraoxonase/arylesterase. Evidence for one esterase catalyzing both activities. *Drug Metab Disposition*. 1991; 19:100–6.
36. La Du BN, Aviram M, Billecke S, Navab M, Primo-Parmo S, Sorenson RC, et al. On the physiological role(s) of the paraoxonases. *Chem Biol Interact*. 1999; 119–120:379–88.
37. Durrington PN, Mackness B, Mackness MI. Paraoxonase and atherosclerosis. *Arterio Thromb Vasc Biol*. 2001; 21:473–80.
38. Purcell K, Schembri M, Frazier LM, Rall MJ, Shen S, Croughan M, et al. Asian ethnicity is associated with reduced pregnancy outcomes after assisted reproductive technology. *Fertil Steril*. 2007; 87:297–302. [PubMed: 17081529]
39. Langen ES, Shahine LK, Lamb JD, Lathi RB, Milki AA, Fujimoto VY, et al. Asian ethnicity and poor outcomes after in vitro fertilization blastocyst transfer. *Obstet Gynecol*. 2010; 115:591–6. [PubMed: 20177291]
40. Tiboni GM, Bucciarelli T, Giampietro F, Sulpizio M, Di Ilio C. Influence of cigarette smoking on vitamin E, vitamin A, β -carotene and lycopene concentrations in human pre-ovulatory follicular fluid. *Int J Immunopathol Pharmacol*. 2004; 17:389–93. [PubMed: 15461873]
41. Palan PR, Cohen BL, Barad DH, Romney SL. Effects of smoking on the levels of antioxidant beta carotene, alpha tocopherol and retinol in human ovarian follicular fluid. *Gynecol Obstet Invest*. 1995; 39:43–6. [PubMed: 7890252]

42. Garrison RJ, Kannel WB, Feinleib M, Castelli WP, McNamara PM, Padgett SJ. Cigarette smoking and HDL cholesterol. The Framingham Offspring study. *Atherosclerosis*. 1978; 30:17–25. [PubMed: 209795]
43. Pacella L, Zander-Fox DL, Armstrong DT, Lane M. Women with reduced ovarian reserve or advanced maternal age have an altered follicular environment. *Fertil Steril*. 2012; 98:986–94. e4. [PubMed: 22818290]
44. Brizzi P, Dessole S, Tonolo G, Capobianco G, Milia L, Puddu L, et al. Effect of ovarian stimulation on plasma lipid and apolipoprotein concentrations in a population of infertile women undergoing IVF/embryo transfer. *Reprod Biomed Online*. 2003; 7:309–12. [PubMed: 14653889]
45. Ya ar HY, Ertugrul O. Clomiphene citrate-induced severe hypertriglyceridemia. *Fertil Steril*. 2009; 92:396, e7–e8. [PubMed: 19380127]
46. Van Blerkom J, Antczak M, Schrader R. The developmental potential of the human oocyte is related to the dissolved oxygen content of follicular fluid: Association with vascular endothelial growth factor levels and perfollicular blood flow characteristics. *Hum Reprod*. 1997; 12:1047–55. [PubMed: 9194664]
47. Van Blerkom J. The influence of intrinsic and extrinsic factors on the developmental potential and chromosomal normality of the human oocyte. *J Soc Gynecol Investig*. 1996; 3:3–11.
48. Shweiki D, Itin A, Soffer D, Keshet E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature*. 1992; 359:843–5. [PubMed: 1279431]
49. Lintsen AME, Eijkemans MJC, Hunault CC, Bouwmans CAM, Hakkaart L, Habbema JDF, et al. Predicting ongoing pregnancy chances after IVF and ICSI: A national prospective study. *Hum Reprod*. 2007; 22:2455–62. [PubMed: 17636281]
50. Taioli E, Kinney P, Zhitkovich A, Fulton H, Voitkun V, Cosma G, et al. Application of reliability models to studies of biomarker validation. *Environ Health Perspect*. 1994; 102:306–9. [PubMed: 8033872]
51. Schulte, PA.; Rothman, N.; Schottenfeld, D. Design considerations in molecular epidemiology. In: Schulte, PA.; Perera, FP., editors. *Molecular Epidemiology: Principles and Practices*. San Diego: Academic Press; 1993. p. 159-98.
52. Lachin JM. The role of measurement reliability in clinical trials. *Clinical trials (London, England)*. 2004; 1:553–66.
53. Leroy JLMR, Vanholder T, Delanghe JR, Opsomer G, Van Soom A, Bols PEJ, et al. Metabolite and ionic composition of follicular fluid from different-sized follicles and their relationship to serum concentrations in dairy cows. *Anim Reprod Sci*. 2004; 80:201–11. [PubMed: 15036497]
54. Nandi S, Kumar VG, Manjunatha BM, Gupta PSP. Biochemical composition of ovine follicular fluid in relation to follicle size. *Dev Growth Differ*. 2007; 49:61–6. [PubMed: 17227345]
55. Browne RW, Bloom MS, Schisterman EF, Hovey K, Trevisan M, Wu C, et al. Analytical and biological variation of biomarkers of oxidative stress during the menstrual cycle. *Biomarkers*. 2008; 13:160–83. [PubMed: 18270869]
56. Barbieri, RL.; Hornstein, MD. Assisted reproduction. In: Strauss, JF.; Barbieri, RL., editors. *Yen and Jaffe's Reproductive Endocrinology: Physiology, Pathophysiology, and Clinical Management*. Philadelphia: Elsevier Saunders; 2004. p. 839-73.
57. Katz P, Showstack J, Smith JF, Nachtigall RD, Millstein SG, Wing H, et al. Costs of infertility treatment: Results from an 18-month prospective cohort study. *Fertil Steril*. 2011; 95:915–21. [PubMed: 21130988]

Table 1Distribution of demographic factors and clinical factors for *in vitro* fertilization patients.

Factor	n	Mean/SD (%)
Age (years)	171	37.3/4.2
BMI (kg/m ²)	171	24.4/4.6
Race ^a		
non-Asian	115	(71.0)
Asian	47	(29.0)
Cigarette smoking ^b		
Never	146	(88.5)
Ever	19	(11.5)
Diagnosis		
Male factor	62	(36.3)
Unexplained ^c	48	(28.1)
Female factor-non DOR ^d	30	(17.5)
Female factor- DOR	27	(15.8)
PGD-only	4	(2.3)
COS protocol		
Lupron down regulated	115	(67.3)
Antagonist	42	(24.6)
Flare	14	(8.2)

^a n=9 missing values;^b n=6 missing values;^c includes n=1 recurrent pregnancy loss;^d includes n=10 endometriosis, n=10 tubal factor, n=7 polycystic ovary syndrome and n=3 anovulation.

BMI, body mass index; COS, controlled ovarian stimulation; DOR, diminished ovarian reserve; PGD, pre-implantation genetic diagnosis; Max, maximum; Min, minimum; SD, standard deviation.

Characteristics of biological variability for concentrations of HDL-particle associated follicular fluid analytes sampled from *in vitro* fertilization patients (n=171).

Table 2

Follicular fluid analytes	Mean	SD	CV	σ^2_T	σ^2_B	σ^2_F	σ^2_A	σ^2_{F+A}	ICC
Group I:									
HDL-cholesterol (mg/dL)	26.87	1.36	5.07	0.096	66.37	31.72	1.90	0.48	0.66
Phospholipids (mg/dL)	71.49	1.31	1.84	0.074	62.96	34.84	2.20	0.55	0.63
Triglycerides (mg/dL)	7.91	1.78	22.52	0.338	82.62	15.57	1.81	0.19	0.83
Arylesterase (kIU/L)	99.51	1.42	1.43	0.124	77.89	20.63	1.48	0.26	0.78
Paraoxonase (IU/L)	78.97	1.66	2.10	0.258	89.65	9.66	0.69	0.11	0.90
ApoA-1 (mg/dL)	89.20	1.32	1.48	0.077	64.96	33.08	1.96	0.51	0.65
ApoA-2 (mg/dL)	26.00	1.33	5.13	0.083	44.66	54.00	1.35	1.21	0.45
Group II:									
Free cholesterol (mg/dL)	2.07	1.26	60.73	0.054	55.56	44.44	-	0.80	0.56
Cholesteryl palmitate (mg/dL)	3.45	1.37	39.63	0.096	35.44	64.56	-	1.82	0.35
Cholesteryl oleate (mg/dL)	7.84	1.31	16.67	0.072	65.14	34.86	-	0.54	0.65
Cholesteryl linoleate (mg/dL)	12.55	1.31	10.42	0.072	63.67	36.33	-	0.57	0.64
Cholesteryl arachidonate (mg/dL)	6.06	0.33	21.97	0.083	72.15	27.85	-	0.39	0.72
Retinol (μ g/mL)	1.34	1.07	79.35	0.004	69.05	30.95	-	0.45	0.69
β -carotene (μ g/mL)	1.05	1.04	98.65	0.002	94.41	5.59	-	0.06	0.94
β -cryptoxanthin (μ g/mL)	1.04	1.04	99.51	0.001	96.79	3.21	-	0.03	0.97
α -tocopherol (μ g/mL)	3.47	1.27	36.68	0.058	69.28	30.72	-	0.44	0.69
γ -tocopherol (μ g/mL)	1.19	1.11	93.38	0.011	81.65	18.35	-	0.22	0.82
Lutein/zeaxanthin (μ g/mL)	1.07	1.03	96.70	0.001	92.91	7.09	-	0.08	0.93
Lycopene (μ g/mL)	1.07	1.03	96.24	0.001	86.41	13.59	-	0.16	0.86

NOTE: Geometric means and standard deviations (SD) presented. All values were log-transformed for the analyses. For Group II analytes, variability due to analytic factors was captured in conjunction with variability between-follicles.

ApoA-1, apolipoprotein A-1; ApoA-2, apolipoprotein A-2; CI, confidence interval; CV, coefficient of variation; HDL, high-density lipoprotein; ICC, intraclass correlation coefficient; σ^2_A , variability attributed to analytic factors; σ^2_B , variability between-women; σ^2_F , variability between-follicles.

Table 3

Characteristics of biological variability for concentrations of HDL-particle associated follicular fluid analytes sampled from contralateral follicles collected from *in vitro* fertilization patients, by demographic factors.

Follicular fluid analytes	Age (years)					
	<35 (n=30)			35 (n=95)		
	% σ^2_B	% σ^2_F	% σ^2_A	% σ^2_B	% σ^2_F	% σ^2_A
Group I:						
HDL-cholesterol	56.94	41.58 ^a	1.48	68.58	29.11 ^a	2.31
Phospholipids	55.49	43.11 ^a	1.39	66.24	31.20 ^a	2.56
Triglycerides	56.55	41.74 ^a	1.71	85.91	12.27 ^b	1.82
Arylesterase	82.22	16.32 ^a	1.46	78.18	20.41 ^a	1.41
Paraoxonase	86.88	12.23 ^a	0.89	90.22	9.08 ^a	0.70
ApoA-1	53.16	45.13 ^a	1.70	66.23	31.70 ^a	2.08
ApoA-2	42.88	55.80 ^a	1.32	44.78	54.05 ^a	1.18
Group II:						
Free cholesterol	47.41	52.59 ^a	-	54.63	45.37 ^a	-
Cholesteryl palmitate	14.72	85.28 ^a	-	39.93	60.07 ^a	-
Cholesteryl oleate	60.08	39.92 ^a	-	65.16	34.84 ^a	-
Cholesteryl linoleate	49.52	50.48 ^a	-	65.51	34.49 ^a	-
Cholesteryl arachidonate	67.50	32.50 ^a	-	73.78	26.22 ^a	-
Retinol	61.42	38.58 ^a	-	72.20	27.80 ^a	-
β -carotene	85.75	14.25 ^a	-	95.97	4.03 ^b	-
β -cryptoxanthin	91.42	8.58 ^a	-	98.18	1.82 ^b	-
α -tocopherol	62.41	37.59 ^a	-	72.48	27.52 ^a	-
γ -tocopherol	52.91	47.09 ^a	-	91.33	8.67 ^b	-
Lutein/zeaxanthin	89.24	10.76 ^a	-	93.65	6.35 ^a	-

Follicular fluid analytes	Age (years)					
	<35 (n=30)		35 (n=95)		30 (n=10)	
	%σ ² _B	%σ ² _F	%σ ² _A	%σ ² _B	%σ ² _F	%σ ² _A
Lycopene	87.85	12.15 ^a	-	85.71	14.29 ^a	-

Follicular fluid analytes	BMI (kg/m ²)					
	<25 (n=86)		25 - 29 (n=30)		30 (n=10)	
	%σ ² _B	%σ ² _F	%σ ² _A	%σ ² _B	%σ ² _F	%σ ² _A
Group I:						
HDL-cholesterol	64.86	33.14 ^a	2.01	60.06	36.91 ^a	3.03
Phospholipids	64.75	32.63 ^a	2.62	50.90	46.76 ^a	2.34
Triglycerides	80.24	17.30 ^a	2.47	84.49	14.83 ^{a,b}	0.68
Arylesterase	80.62	18.03 ^a	1.36	70.04	28.53 ^a	1.43
Paraoxonase	88.67	10.47 ^a	0.86	90.24	9.27 ^a	0.49
ApoA-1	63.76	34.54 ^a	1.70	55.55	42.13 ^a	2.32
ApoA-2	42.04	56.84 ^a	1.12	43.62	54.89 ^a	1.49
Group II:						
Free cholesterol	56.18	43.82 ^a	-	46.46	53.54 ^a	-
Cholesteryl palmitate	39.58	60.42 ^a	-	2.94	97.06 ^b	-
Cholesteryl oleate	65.12	34.88 ^a	-	47.69	52.31 ^a	-
Cholesteryl linoleate	63.17	36.84 ^a	-	46.41	53.59 ^a	-
Cholesteryl arachidonate	70.05	29.95 ^a	-	74.54	25.46 ^a	-
Retinol	74.36	25.64 ^a	-	46.56	53.44 ^b	-
β-carotene	94.30	5.70 ^a	-	93.16	6.84 ^a	-
β-cryptoxanthin	97.34	2.66 ^a	-	96.12	3.88 ^a	-
α-tocopherol	65.19	34.81 ^a	-	81.13	18.87 ^a	-
γ-tocopherol	78.05	21.95 ^a	-	86.50	13.50 ^a	-
Lutein/zeaxanthin	92.88	7.12 ^a	-	92.39	7.61 ^a	-

Follicular fluid analytes	BMI (kg/m ²)											
	<25 (n=86)				25 – 29 (n=30)				30 (n=10)			
	%σ ² _B	%σ ² _F	%σ ² _A	%σ ² _F	%σ ² _B	%σ ² _F	%σ ² _A	%σ ² _F	%σ ² _B	%σ ² _F	%σ ² _A	%σ ² _F
Lycopene	85.56	14.44 ^a	-	81.55	18.45 ^a	-	93.53	6.47 ^a	-	-	-	-

Follicular fluid analytes	Race											
	Non-Asian (n=85)				Asian (n=35)				Cigarette smoking			
	Never (n=105)		Ever (n=15)		Never (n=105)		Ever (n=15)		Never (n=105)		Ever (n=15)	
%σ ² _B	%σ ² _F	%σ ² _A	%σ ² _F	%σ ² _B	%σ ² _F	%σ ² _A	%σ ² _F	%σ ² _B	%σ ² _F	%σ ² _A	%σ ² _F	
Group I:												
HDL-cholesterol	63.08	35.38 ^a	1.54	69.72	26.44 ^a	3.84	60.89	37.05 ^a	2.06	74.08	22.97 ^a	2.95
Phospholipids	63.21	34.94 ^a	1.85	60.50	34.80 ^a	4.70	58.89	38.61 ^a	2.50	70.48	28.08 ^a	1.43
Triglycerides	80.92	17.26 ^a	1.82	76.13	21.73 ^a	2.14	80.90	17.33 ^a	1.76	85.70	12.43 ^a	1.87
Arylesterase	76.09	22.57 ^a	1.33	89.68	8.98 ^b	1.35	75.39	23.12 ^a	1.49	84.67	14.11 ^a	1.21
Paraoxonase	86.48	12.58 ^a	0.94	93.39	6.15 ^b	0.46	89.74	9.45 ^a	0.81	88.28	10.91 ^a	0.81
ApoA-1	62.57	35.76 ^a	1.67	60.80	35.76 ^a	3.44	59.50	38.40 ^a	2.10	70.29	28.28 ^a	1.43
ApoA-2	57.38	41.49 ^a	1.13	0.00	98.74 ^a	1.26	52.35	46.15 ^a	1.49	0.00	99.26 ^b	0.74
Group II:												
Free cholesterol	48.43	51.57 ^a	-	61.28	38.72 ^a	-	46.85	53.15 ^a	-	82.75	17.25 ^b	-
Cholesteryl palmitate	34.10	65.90 ^a	-	34.98	65.02 ^a	-	28.55	71.45 ^a	-	52.77	47.23 ^a	-
Cholesteryl oleate	63.96	36.04 ^a	-	63.63	36.37 ^a	-	59.97	40.03 ^a	-	78.33	21.67 ^a	-
Cholesteryl linoleate	58.87	41.13 ^a	-	65.08	34.92 ^a	-	57.76	42.24 ^a	-	74.66	25.34 ^a	-
Cholesteryl arachidonate	69.33	30.67 ^a	-	77.56	22.44 ^a	-	65.09	34.91 ^a	-	88.05	11.95 ^a	-
Retinol	61.71	38.29 ^a	-	85.56	14.44 ^b	-	67.54	32.46 ^a	-	61.10	38.90 ^a	-
β-carotene	88.92	11.08 ^a	-	97.08	2.92 ^b	-	96.17	3.83 ^a	-	94.49	5.51 ^a	-
β-cryptoxanthin	96.15	3.85 ^a	-	99.05	0.95 ^b	-	96.99	3.01 ^a	-	96.77	3.23 ^a	-
α-tocopherol	64.91	35.09 ^a	-	88.27	11.73 ^b	-	68.71	31.29 ^a	-	77.01	22.99 ^a	-
γ-tocopherol	76.88	23.12 ^a	-	89.87	10.13 ^b	-	89.91	10.09 ^a	-	41.19	58.81 ^b	-
Lutein/zeaxanthin	92.43	7.57 ^a	-	92.86	7.14 ^a	-	92.64	7.36 ^a	-	91.56	8.44 ^a	-

Follicular fluid analytes	Race						Cigarette smoking					
	Non-Asian (n=85)		Asian (n=35)		Never (n=105)		Ever (n=15)		Never (n=105)		Ever (n=15)	
	% σ^2_B	% σ^2_F	% σ^2_A	% σ^2_B	% σ^2_F	% σ^2_A	% σ^2_B	% σ^2_F	% σ^2_A	% σ^2_B	% σ^2_F	% σ^2_A
Lycopene	79.71	20.29 ^a	-	94.06	5.94 ^b	-	86.44	13.56 ^a	-	84.14	15.86 ^a	-

NOTE: All values were natural log-transformed prior to analysis. For Group II analytes, variability due to analytic factors was captured in conjunction with variability between-follicles. Sample sizes varied for groups due to missing demographic data and limited follicular fluid available for some analyses; detailed sample counts are provided in Supplemental Table 1.

^{a, b} Different letters in superscript following % σ^2_F values indicate $P < 0.05$ for difference between groups; for example there is no significant difference across groups for HDL-cholesterol between races, yet arylesterase is significantly different between Asian and non-Asian women.

ApoA-1, apolipoprotein A-1; ApoA-2, apolipoprotein A-2; BMI, body mass index; HDL, high-density lipoprotein; σ^2_A , variability attributed to analytic factors; σ^2_B , variability between-women; σ^2_F , variability between-follicles.

Table 4

Characteristics of biological variability for concentrations of HDL-particle associated follicular fluid analytes sampled from contralateral follicles collected from 171 *in vitro* fertilization patients, by clinical factors.

Follicular Fluid analytes	Diagnosis ^a												
	Male factor (n=38)			Female factor (n=23)			DOR (n=18)			Unexplained (n=45)			
	%σ ² _B	%σ ² _F	%σ ² _A	%σ ² _B	%σ ² _F	%σ ² _A	%σ ² _B	%σ ² _F	%σ ² _A	%σ ² _B	%σ ² _F	%σ ² _A	
Group I:													
HDL-cholesterol	53.47	43.97 ^a	2.56	63.73	35.79 ^a	0.48	90.06	9.40 ^b	0.54	68.27	28.59 ^a	3.14	
Phospholipids	44.36	53.53 ^a	2.11	61.62	37.08 ^a	1.29	88.48	7.31 ^b	4.21	68.40	28.73 ^a	2.87	
Triglycerides	71.53	26.93 ^a	1.54	86.03	12.91 ^{a,b}	1.06	90.30	7.57 ^b	2.13	79.96	17.82 ^{a,b}	2.23	
Arylesterase	62.72	35.22 ^a	2.06	80.16	18.43 ^a	1.42	97.96	1.30 ^b	0.75	80.28	18.33 ^a	1.39	
Paraoxonase	84.80	14.09 ^a	1.11	87.54	12.12 ^a	0.34	98.65	0.90 ^b	0.44	90.36	8.74 ^a	0.90	
ApoA-1	42.46	54.82 ^a	2.73	66.83	31.89 ^a	1.27	94.14	4.35 ^b	1.52	65.65	32.34 ^a	2.01	
ApoA-2	31.51	66.88 ^{a,b}	1.60	56.54	42.41 ^{a,c}	1.05	0.00	99.12 ^b	0.88	66.59	32.11 ^c	1.30	
Group II:													
Free cholesterol	34.06	65.94 ^a	-	57.13	42.87 ^a	-	58.25	41.75 ^a	-	59.38	40.62 ^a	-	
Cholesteryl palmitate	10.98	89.02 ^a	-	32.33	67.67 ^{a,b}	-	67.63	32.37 ^b	-	43.43	56.57 ^{a,b}	-	
Cholesteryl oleate	49.55	50.45 ^a	-	59.00	41.00 ^a	-	59.30	40.70 ^a	-	71.35	28.65 ^a	-	
Cholesteryl linoleate	56.14	43.86 ^a	-	52.60	47.40 ^a	-	67.42	32.58 ^a	-	68.98	31.01 ^a	-	
Cholesteryl arachidonate	68.06	31.94 ^a	-	61.73	38.27 ^a	-	77.29	22.71 ^a	-	78.29	21.71 ^a	-	
Retinol	59.05	40.95 ^a	-	56.26	43.74 ^a	-	80.41	19.59 ^a	-	77.49	22.51 ^a	-	
β-carotene	94.14	5.86 ^a	-	92.73	7.27 ^a	-	91.27	8.73 ^a	-	94.79	5.21 ^a	-	
β-cryptoxanthin	95.68	4.32 ^a	-	95.17	4.83 ^a	-	94.85	5.15 ^a	-	99.03	0.97 ^b	-	
α-tocopherol	71.58	28.42 ^{a, b}	-	49.43	50.57 ^a	-	76.87	23.13 ^{a,b}	-	83.51	16.49 ^b	-	
γ-tocopherol	90.96	9.04 ^a	-	58.44	41.56 ^b	-	88.80	11.20 ^a	-	89.80	10.20 ^a	-	
Lutein/zeaxanthin	89.22	10.78 ^a	-	90.63	9.37 ^a	-	80.72	19.28 ^a	-	95.87	4.13 ^b	-	

Follicular Fluid analytes	Diagnosis ^a											
	Male factor (n=38)			Female factor (n=23)			DOR (n=18)			Unexplained (n=45)		
	%σ ² _B	%σ ² _F	%σ ² _A	%σ ² _B	%σ ² _F	%σ ² _A	%σ ² _B	%σ ² _F	%σ ² _A	%σ ² _B	%σ ² _F	%σ ² _A
Lycopene	85.75	14.25 ^a	-	91.26	8.74 ^a	-	86.36	13.64 ^a	-	84.39	15.61 ^a	-

Follicular Fluid analytes	COS Protocol																	
	Lupron down regulated (n=84)						Antagonist (n=34)						Flare (n=7)					
	%σ ² _B	%σ ² _F	%σ ² _A	%σ ² _B	%σ ² _F	%σ ² _A	%σ ² _B	%σ ² _F	%σ ² _A	%σ ² _B	%σ ² _F	%σ ² _A	%σ ² _B	%σ ² _F	%σ ² _A			
Group I:																		
HDL-cholesterol	63.24	34.41 ^a	2.35	65.22	33.22 ^a	1.56	94.72	4.37 ^b	0.91									
Phospholipids	58.75	39.36 ^a	1.88	76.52	20.55 ^a	2.92	90.60	3.53 ^a	5.87									
Triglycerides	81.41	16.67 ^a	1.93	79.71	18.69 ^a	1.61	94.90	3.94 ^a	1.17									
Arylesterase	76.57	22.06 ^a	1.37	85.55	12.29 ^{a, b}	2.16	97.92	1.59 ^b	0.49									
Paraoxonase	87.10	12.18 ^a	0.72	93.74	5.13 ^b	1.13	98.37	1.51 ^b	0.11									
ApoA-1	58.30	39.67 ^a	2.03	76.41	21.43 ^a	2.16	96.06	3.16 ^b	0.79									
ApoA-2	52.39	46.43 ^a	1.18	79.44	18.58 ^b	1.98	0.00	99.71 ^c	0.29									
Group II:																		
Free cholesterol	64.07	35.93 ^a	-	27.39	72.61 ^b	-	62.93	37.07 ^{a, b}	-									
Cholesteryl palmitate	30.62	69.38 ^a	-	32.99	68.01 ^a	-	75.27	24.73 ^a	-									
Cholesteryl oleate	63.84	36.16 ^a	-	57.50	42.50 ^a	-	63.06	36.94 ^a	-									
Cholesteryl linoleate	60.65	39.35 ^a	-	58.41	41.59 ^a	-	80.86	19.14 ^a	-									
Cholesteryl arachidonate	72.07	27.93 ^a	-	67.86	32.14 ^a	-	72.31	27.69 ^a	-									
Retinol	64.82	35.18 ^a	-	74.60	25.40 ^a	-	87.86	12.14 ^a	-									
β-carotene	91.10	8.90 ^a	-	97.80	2.20 ^b	-	86.78	13.22 ^a	-									
β-cryptoxanthin	96.51	3.49 ^a	-	98.45	1.55 ^b	-	99.54	0.46 ^b	-									
α-tocopherol	67.55	32.45 ^a	-	75.75	24.25 ^a	-	84.59	15.41 ^a	-									
γ-tocopherol	78.84	21.16 ^a	-	89.18	10.82 ^b	-	80.52	19.48 ^{a, b}	-									

Follicular Fluid analytes	COS Protocol													
	Lupron down regulated (n=84)						Antagonist (n=34)						Flare (n=7)	
	% σ^2_B	% σ^2_F	% σ^2_A	% σ^2_B	% σ^2_F	% σ^2_A	% σ^2_B	% σ^2_F	% σ^2_A	% σ^2_B	% σ^2_F	% σ^2_B	% σ^2_F	
Lutein/zeaxanthin	92.35	7.65 ^a	-	91.58	8.42 ^a	-	95.44	4.56 ^a	-	95.44	4.56 ^a	-	-	
Lycopene	84.38	15.62 ^a	-	88.57	11.43 ^{a,b}	-	96.54	3.46 ^b	-	96.54	3.46 ^b	-	-	

NOTE: All values were natural log-transformed prior to analysis. For Group II analytes, variability due to analytic factors was captured in conjunction with variability between-follicles. Sample sizes for groups varied due to limited follicular fluid available for some analyses; detailed sample counts are provided in Supplemental Table 2.

^a, ^b, ^c Different letters in superscript following % σ^2_F values indicate $P < 0.05$ for difference between groups; for example there is no significant difference across COS-protocol groups for HDL-cholesterol, yet free cholesterol is significantly different between Lupron down regulated and antagonist, but not flare.

DOR, diminished ovarian reserve; COS, controlled ovarian stimulation; ApoA-1, apolipoprotein A-1; ApoA-2, apolipoprotein A-2; σ^2_A , variability attributed to analytic factors; σ^2_B , variability between-women; σ^2_F , variability between-follicles.