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

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## 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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**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 $O_2$ (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 ($E_2$). When a sufficient number of follicles ≥17 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 ≥17 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 (*PON1*) 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 cholesteryls 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 μg/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 *PON1* 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 cholesteryls 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/$m^2$) (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 $E_2$ 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/m$^2$ those with BMI ≥30 kg/m$^2$ 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 ≥30 kg/m$^2$ than for <25 kg/m$^2$. Paraoxonase was higher in Asians (δ=28.91 IU/L; P<.0001) whereas cholesteryl arachidonate was lower (δ=0.60 mg/dL; P=00.04), 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 ≥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 ≥30 kg/m$^2$ 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≤ BMI <30 kg/m$^2$ 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 hypertriglycedemia 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 $O_2$ 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 $O_2$ 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 *PON1* 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/m$^2$ included with BMI <25 kg/m$^2$, BMI >40 kg/m$^2$ included with BMI ≥30 kg/m$^2$) 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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## Table 1

Distribution 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.

**Table 2**

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

| Follicular fluid analytes | Mean | SD | CV | $\sigma^2_T$ | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ | $\%\sigma^2_F$:$\%\sigma^2_B$ | 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 (μg/mL) | 1.34 | 1.07 | 79.35 | 0.004 | 69.05 | 30.95 | - | 0.45 | 0.69 |
| β-carotene (μg/mL) | 1.05 | 1.04 | 98.65 | 0.002 | 94.41 | 5.59 | - | 0.06 | 0.94 |
| β-cryptoxanthin (μg/mL) | 1.04 | 1.04 | 99.51 | 0.001 | 96.79 | 3.21 | - | 0.03 | 0.97 |
| α-tocopherol (μg/mL) | 3.47 | 1.27 | 36.68 | 0.058 | 69.28 | 30.72 | - | 0.44 | 0.69 |
| γ-tocopherol (μg/mL) | 1.19 | 1.11 | 93.38 | 0.011 | 81.65 | 18.35 | - | 0.22 | 0.82 |
| Lutein/zeaxanthin (μg/mL) | 1.07 | 1.03 | 96.70 | 0.001 | 92.91 | 7.09 | - | 0.08 | 0.93 |
| Lycopene (μg/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; $\sigma^2_A$, variability attributed to analytic factors; $\sigma^2_B$, variability between-women; $\sigma^2_F$, 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) | | |
|---|---|---|---|---|---|---|
| | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ |
| 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) | | |
| | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ |
| 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) | | |
| | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ |
| Group I: | | | | | | | | | |
| HDL-cholesterol | 64.86 | 33.14[a] | 2.01 | 60.06 | 36.91[a] | 3.03 | 83.04 | 15.97[a] | 0.99 |
| Phospholipids | 64.75 | 32.63[a] | 2.62 | 50.90 | 46.76[a] | 2.34 | 65.01 | 34.43[a] | 0.55 |
| Triglycerides | 80.24 | 17.30[a] | 2.47 | 84.49 | 14.83[a,b] | 0.68 | 58.59 | 40.8 [b] | 0.56 |
| Arylesterase | 80.62 | 18.03[a] | 1.36 | 70.04 | 28.53[a] | 1.43 | 88.09 | 9.13[a] | 2.77 |
| Paraoxonase | 88.67 | 10.47[a] | 0.86 | 90.24 | 9.27[a] | 0.49 | 95.36 | 4.18[a] | 0.46 |
| ApoA-1 | 63.76 | 34.54[a] | 1.70 | 55.55 | 42.13[a] | 2.32 | 84.51 | 10.63[a] | 4.86 |
| ApoA-2 | 42.04 | 56.84[a] | 1.12 | 43.62 | 54.89[a] | 1.49 | 74.41 | 22.39[a] | 3.20 |
| Group II: | | | | | | | | | |
| Free cholesterol | 56.18 | 43.82[a] | - | 46.46 | 53.54[a] | - | 17.75 | 82.25[a] | - |
| Cholesteryl palmitate | 39.58 | 60.42[a] | - | 2.94 | 97.06[b] | - | 63.65 | 36.35[a] | - |
| Cholesteryl oleate | 65.12 | 34.88[a] | - | 47.69 | 52.31[a] | - | 66.39 | 33.61[a] | - |
| Cholesteryl linoleate | 63.17 | 36.84[a] | - | 46.41 | 53.59[a] | - | 68.29 | 31.71[a] | - |
| Cholesteryl arachidonate | 70.05 | 29.95[a] | - | 74.54 | 25.46[a] | - | 81.71 | 18.29[a] | - |
| Retinol | 74.36 | 25.64[a] | - | 46.56 | 53.44[b] | - | 58.57 | 41.43[a,b] | - |
| β-carotene | 94.30 | 5.70[a] | - | 93.16 | 6.84[a] | - | 93.72 | 6.28[a] | - |
| β-cryptoxanthin | 97.34 | 2.66[a] | - | 96.12 | 3.88[a] | - | 85.54 | 14.46[b] | - |
| α-tocopherol | 65.19 | 34.81[a] | - | 81.13 | 18.87[a] | - | 74.71 | 25.29[a] | - |
| γ-tocopherol | 78.05 | 21.95[a] | - | 86.50 | 13.50[a] | - | 91.40 | 8.60[a] | - |
| Lutein/zeaxanthin | 92.88 | 7.12[a] | - | 92.39 | 7.61[a] | - | 69.39 | 30.63[b] | - |

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

| Follicular fluid analytes | Race | | | | | | Cigarette smoking | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Non-Asian (n=85) | | | Asian (n=35) | | | Never (n=105) | | | Ever (n=15) | | |
| | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ |
| 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) | | |
| | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ |
| 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 $\%\sigma^2_F$ 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; $\sigma^2_A$, variability attributed to analytic factors; $\sigma^2_B$, variability between-women; $\sigma^2_F$, 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) | | |
| | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ |
| 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) | | |
| | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ |
| 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) | | |
| | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ |
| 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) | | |
| | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ | $\%\sigma^2_B$ | $\%\sigma^2_F$ | $\%\sigma^2_A$ |
| Lutein/zeaxanthin | 92.35 | 7.65[a] | - | 91.58 | 8.42[a] | - | 95.44 | 4.56[a] | - |
| Lycopene | 84.38 | 15.62[a] | - | 88.57 | 11.43[a,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 $\%\sigma^2_F$ 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; $\sigma^2_A$, variability attributed to analytic factors; $\sigma^2_B$, variability between-women; $\sigma^2_F$, variability between-follicles.