Supplemental Methods File

Statistical analysis of measurement variability sources

Using two-stage nested ANOVA we characterized sources of variability among participants with a 'balanced' data set, including four FF determinations for group I analytes and two FF determinations for group II analytes (Supplemental Figure 1). We defined variability as due to factors between-women and between-follicles (contralateral), and due to analytic factors. Models were specified as $Y_{ijk} = \mu + sub_i + follicle_{j(i)} + e_{k(ji)}$; where Y_{ijk} describes an analyte value for the *k*th determination (k=1, 2) collected from the *j*th follicle (j=1, 2) from the *i*th woman (i=1, …, 177), μ describes the grand mean for an analyte, sub_i describes the random effect of the *i*th woman on the grand mean, $follicle_{j(i)}$ describes the random effect of the *j*th follicle nested in the *i*th woman, and $e_{k(ji)}$ describes the random effect of the *k*th determination nested within the *j*th follicle sampled from the *i*th woman. Under this model specification, sources of variability for Group I analytes were characterized in terms of sources between-women (σ^2_B), between-follicles (σ^2_F) and for analytic factors (σ^2_A). For Group II analytes, we were unable to separate variability due to analytic factors from sources between-follicles.

Total variance for an analyte was defined as $\sigma^2_T = \sigma^2_B + \sigma^2_F + \sigma^2_A$ for Group I analytes, and $\sigma^2_T = \sigma^2_B + \sigma^2_F$ for Group II analytes; relative contributions for each source to the total were calculated as proportions multiplied by 100 ($\%\sigma^2_B$, $\%\sigma^2_F$ and $\%\sigma^2_A$, respectively). The relative contributions for each source to total measurement variability were also characterized by demographic and clinical factors; we used F-tests to assess the significance of σ^2_F for analytes stratified by these factors. To test for differences between σ^2_F across demographic and clinical groups, we assessed overlap of 84% confidence intervals, which approximates a statistical test with type-1 error rate

(α) equal to 0.05 (1). We also characterized ratios of variability between-follicles to between-women ($\%\sigma^2_F$:$\%\sigma^2_B$), to provide an index of the relative contributions of these sources to the total, analogous to the index of individuality (2). The higher the value of this ratio, the more an analyte appeared to depend upon the individual follicle, while lower values were more dependent on the individual woman. Coefficients of variation (CV) were calculated as $CV = \sqrt{\sigma^2_l} / \bar{x}_l$, where $\sqrt{\sigma^2_l}$ represents the total variance and $\bar{x}_l$ represents the mean value for an analyte. Intraclass correlation coefficients (ICC) were estimated as σ^2_B / σ^2_T, with 95% confidence intervals (CIs) estimated using the inverse tan transformation of Smith's variance (3).

References

1. Julious SA. Using confidence intervals around individual means to assess statistical significance between two means. Pharm Stat 2004;3:217-22.

2. Fraser CG, Harris EK, Petersen PH. Generation and application of data on biological variation in clinical chemistry. Crit Rev Clin Lab Sci 1989;27:409-30.

3. Lachin JM. The role of measurement reliability in clinical trials. Clinical trials (London, England) 2004;1:553-66.

Supplemental Tables File

Supplemental Table 1. Sample sizes for biologic variability analysis of HDL-particle associated follicular fluid analytes, by demographic factors.

	Age (years)		BMI (kg/m^2)			Race		Cigarette smoking	
Follicular fluid analytes	<35	≥35	<25	25≤BMI<30	≥30	Non-Asian	Asian	Never	Ever
Group I:									
HDL-cholesterol	28	90	83	27	8	78	34	99	14
Phospholipids	28	90	83	27	8	78	34	99	14
Triglycerides	28	90	83	27	8	78	34	99	14
Arylesterase	28	90	83	27	8	78	34	99	14
Paraoxonase	28	90	83	27	8	78	34	99	14
ApoA-1	28	90	83	27	8	78	34	99	14
ApoA-2	27	86	81	25	7	75	33	96	14
Group II:									
Free cholesterol	30	95	85	30	10	85	34	105	15
Cholesteryl palmitate	30	95	85	30	10	85	34	105	15
Cholesteryl oleate	30	95	85	30	10	85	34	105	15
Cholesteryl linoleate	30	95	85	30	10	85	34	105	15
Cholesteryl arachidonate	30	95	85	30	10	85	34	105	15
Retinol	29	93	86	28	8	81	35	103	14
β-carotene	29	93	86	28	8	81	35	103	14
β-cryptoxanthin	29	93	86	28	8	81	35	103	14
α-tocopherol	29	93	86	28	8	81	35	103	14
γ-tocopherol	29	93	86	28	8	81	35	103	14
Lutein/zeaxanthin	29	93	86	28	8	81	35	103	14
Lycopene	29	93	86	28	8	81	35	103	14

NOTE: Sample sizes varied due to missing demographic data and limited follicular fluid available for some analyses.

ApoA-1, apolipoprotein A-1; ApoA-2, apolipoprotein A-2; BMI, body mass index; HDL, high-density lipoprotein.

Supplemental Table 2. Sample sizes for biologic variability analysis of HDL-particle associated follicular fluid analytes, by clinical factors.

	Diagnosis				COS Protocol		
Follicular fluid analytes	Male factor	Female factor	DOR	Unexplained	Lupron down regulated	Antagonist	Flare
Group I:							
HDL-cholesterol	35	23	15	44	82	30	6
Phospholipids	35	23	15	44	82	30	6
Triglycerides	35	23	15	44	82	30	6
Arylesterase	35	23	15	44	82	30	6
Paraoxonase	35	23	15	44	82	30	6
ApoA-1	35	23	15	44	82	30	6
ApoA-2	33	21	15	43	78	29	6
Group II:							
Free cholesterol	38	23	18	44	84	34	7
Cholesteryl palmitate	38	23	18	44	84	34	7
Cholesteryl oleate	38	23	18	44	84	34	7
Cholesteryl linoleate	38	23	18	44	84	34	7
Cholesteryl arachidonate	38	23	18	44	84	34	7
Retinol	37	21	18	45	83	32	7
β-carotene	37	21	18	45	83	32	7
β-cryptoxanthin	37	21	18	45	83	32	7
α-tocopherol	37	21	18	45	83	32	7
γ-tocopherol	37	21	18	45	83	32	7
Lutein/zeaxanthin	37	21	18	45	83	32	7
Lycopene	37	21	18	45	83	32	7

NOTE: Sample sizes varied due to limited follicular fluid available for some analyses.

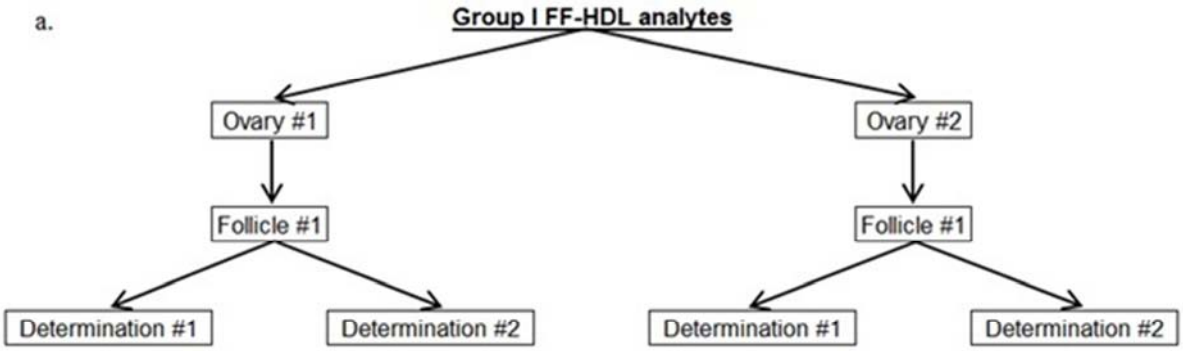
DOR, diminished ovarian reserve; COS, controlled ovarian stimulation; ApoA-1, apolipoprotein A-1; ApoA-2, apolipoprotein A-2; σ^2_A, variability attributed to analytic factors; σ^2_B, variability between-women; σ^2_F, variability between-follicles.

Supplemental Figures File

Supplemental Figure 1. Sampling strategy for the measurement of follicular fluid (FF) high-density lipoprotein (HDL) analytes collected from *in vitro* fertilization patients: a) Group I analytes included HDL-cholesterol, phospholipids, triglycerides, apolipoproteins (ApoA-1 and ApoA-2), arylesterase and paraoxonase, for which two determinations were made per sampled follicle; b) Group II analytes included free cholesterol, cholesteryl palmitate, cholesteryl oleate, cholesteryl linoleate, cholesteryl arachidonate, retinol, β-carotene, β-cryptoxanthin, α-tocopherol, γ-tocopherol, lutein/zeaxanthin and lycopene, for which one determination was made per sampled follicle.

Supplemental Figure 2. Intraclass correlation coefficients (ICCs, 95% confidence intervals (CIs)) for follicular fluid analytes measured in specimens collected from contralateral ovaries, including HDL-cholesterol, phospholipids, triglycerides, arylesterase, paraoxonase, apolipoprotein A-1 (ApoA-1) and A-2 (ApoA-2), free cholesterol, cholesteryl palmitate, cholesteryl oleate, cholesteryl linoleate, cholesteryl arachidonate, retinol, β-carotene, β-cryptoxanthin, α-tocopherol, γ-tocopherol, lutein/zeaxanthin and lycopene.

a.



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