Web Table 1. Comparison of Mean[a] in Semen Parameters Between the First Semen Sample and the Long-Term Averages of Other Samples Among 329 Men (768 Samples) From the Environment and Reproductive Health Study, Boston, Massachusetts, 2005–2014

Semen Parameter	First Sample		Remaining Samples		Difference[b] between First and Remaining Samples		Intraclass Correlation Coefficient	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Ejaculate volume[c], ml	2.7	2.6, 2.9	2.6	2.5, 2.8	0.03	-0.01, 0.06	0.71	0.66, 0.76
Concentration[d], x10^6/ml	54.8	49.8, 60.4	52.9	47.3, 59.1	4%	-5%, 13%	0.76	0.71, 0.80
Total sperm count[d], x10^6	140	127, 155	131	117, 147	7%	-3%, 18%	0.64	0.58, 0.70
Total motility[e],%	45.5	42.3, 48.6	44.1	40.7, 47.5	1.37	-0.91, 3.66	0.66	0.60, 0.72
Progressive motility[c,e], %	22.9	20.6, 25.2	22.0	19.8, 24.4	0.09	-0.08, 0.26	0.64	0.57, 0.70
Morphology[c], %	6.4	6.0, 6.8	6.4	6.0, 6.8	-0.02	-0.10, 0.05	0.64	0.57, 0.70
Total motile count[d], x10^6	53.4	45.5, 62.8	47.8	39.7, 57.6	12%	-4%, 30%	0.72	0.66, 0.77
Total normal count[d], x10^6	5.8	5.3, 6.2	5.9	5.5, 6.3	4%	-9%, 18%	0.73	0.67, 0.77

Abbreviation: CI, confidence interval.

[a] Adjusted for age (<35, ≥35 years), and abstinence time (<2 days, ≥2 to <3 days, ≥3 to <4 days, ≥4 days).

[b] Relative differences (%) were presented for total sperm count, sperm concentration, total motile count and total normal count; absolute differences (unit for corresponding semen parameters) were presented for other semen parameters. For ejaculate volume, progressive motility morphology, data were presented as the difference in square roots of the mean. Estimates were obtained from linear mixed model comparing the mean of the first sample and the mean of the other samples accounting for within-man variation.

[c] Ejaculate volume, progressive motility and morphology was square root transformed, and mean (95% CI) was presented in the original scale by back-transformation.

[d] These semen parameters were log transformed, and mean (with 95% CI) was presented in the original scale by back-transformation

[e] Additionally adjusted for time between semen collection and semen analysis (≤ 30 min, >30 min).

Web Figure 1. Comparison of power to detect differences in sperm concentration and morphology between groups when obtaining two replicates from each man versus one replicate from each man at a given sample size. In a study aimed at comparing the average differences in semen quality between 2 groups of men differing in a given exposure, power =

$$\phi[\frac{\Delta}{\sigma^2\left(\frac{1}{N_1}+\frac{1}{N_2}\right)} - Z_{1-\frac{\alpha}{2}}] = \phi[\frac{\Delta}{(\sigma_b^2+\frac{\sigma_w^2}{K})\cdot\left(\frac{1}{N_1}+\frac{1}{N_2}\right)} - Z_{1-\frac{\alpha}{2}}],$$

where $\sigma^2 = \sigma_b^2 + \frac{\sigma_w^2}{K}$, σ_b^2 = between-man variance, σ_w^2 = within-man variance, N_1 = number of subjects in group1, N_2 = number of subjects in group 2, Δ = difference in semen parameters between group 1 and group 2, and K = number of replicates per subject. Supposed Δ is fixed (0.03 for log concentration and square root of morphology), the 2 groups have equal numbers of men ($N_1 = N_2 = N$), $\alpha = 0.05$, and σ_w^2 and σ_b^2 were estimated using data in the EARTH Study. The figure showed how much power would be gained when we asked for 2 replicates from each man versus one replicate from each man at a given sample size.
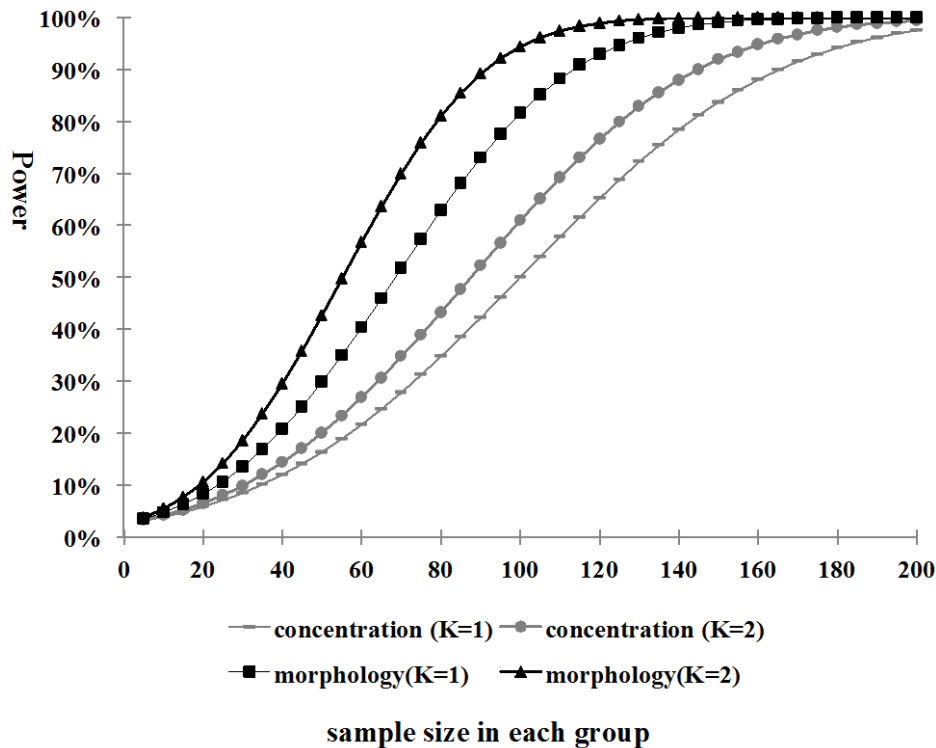
Web Figure 2. Change in power to detect differences in sperm concentration when the number of replicates per man increases for a fixed number of semen sample in each group. In a study aimed at comparing the average differences in semen quality between 2 groups of men with equal sample size but differing in a given exposure, power =

$$\phi[\frac{\Delta}{\sigma^2(\frac{2}{N})} - Z_{1-\frac{\alpha}{2}}] = \phi[\frac{\Delta}{(\sigma_b^2 + \frac{\sigma_w^2}{B/N})(\frac{2}{N})} - Z_{1-\frac{\alpha}{2}}] = \phi[\frac{\Delta}{(\sigma_b^2 * 2/N + \frac{2\sigma_w^2}{B})} - Z_{1-\frac{\alpha}{2}}],$$

where σ_b^2 = between-man variance, σ_w^2 = within-man variance, B = total number of samples, K = number of replicates per man, and N = number of subjects per group. Supposed total number of samples are fixed (i.e., $N \times K = B = 300$ semen samples), $\alpha = 0.05$, and variances were estimated using data in the EARTH Study.