Electronic Supplementary Material (ESM)

Linking sperm length and velocity: the importance of intra-male variation

John L. Fitzpatrick, Francisco Garcia-Gonzalez, Jonathan P. Evans

ESM Table S1. Mean correlation coefficients ($\bar{x}$ r) for the 18 intra-male correlations between sperm morphology measures and two measures of sperm velocity: VAP and VCL. Sperm flagellum length and total length best predicted sperm velocity in our sample. However, we chose to focus our morphological analyses on sperm flagellum length, as it was marginally the best predictor of sperm velocity (highlighted in bold text), and flagellum length makes up 78.26 ± 0.11% of total sperm length in our sample.

Sperm Morphology Measure	VAP	VCL
head length	0.24	0.21
flagellum length	**0.72**	**0.80**
total sperm length	0.71	0.79
flagellum:head length ratio	0.49	0.63

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ESM Table S2: Correlations between sperm flagellum length and sperm swimming velocity, measured using VAP and VCL. Correlations were performed within each male's ejaculate for the 18 males examined. For each correlation we report the number of sperm measured within each male's ejaculate (n), correlation coefficient (*r*), *p*-value, and the 95% confidence limits (CL) generated by bootstrapping the correlation coefficients (10,000 iterations). Regardless of the measure of sperm velocity used in the analyses, within each male's ejaculate the 95% CL for the relationship between flagellum length and sperm velocity did not overlap with zero.

Male		VAP			VCL		
Identity	**n**	***r***	***p***	**CL**	***r***	***p***	**CL**
1	10	0.75	0.01	0.33 - 0.95	0.84	0.002	0.69 - 0.97
2	11	0.62	0.04	0.24 - 0.87	0.73	0.01	0.42 - 0.91
3	11	0.75	<0.01	0.25 - 0.97	0.87	<0.001	0.72 - 0.97
4	21	0.66	<0.001	0.35 - 0.86	0.86	<0.0001	0.70 - 0.95
5	18	0.75	<0.001	0.48 - 0.91	0.60	0.009	0.29 - 0.84
6	14	0.69	<0.01	0.40 - 0.89	0.74	0.003	0.47 - 0.90
7	20	0.71	<0.001	0.53 - 0.87	0.63	0.003	0.33 - 0.85
8	20	0.62	<0.01	0.16 - 0.90	0.70	<0.001	0.36 - 0.89
9	13	0.87	<0.001	0.54 - 0.98	0.68	0.01	0.16 - 0.98
10	20	0.53	0.02	0.15 - 0.84	0.79	<0.0001	0.61 - 0.90
11	20	0.70	<0.001	0.48 - 0.87	0.87	<0.0001	0.76 - 0.96
12	20	0.64	<0.01	0.38 - 0.82	0.78	<0.0001	0.53 - 0.93
13	20	0.81	<0.0001	0.63 - 0.91	0.91	<0.0001	0.83 - 0.96
14	21	0.88	<0.0001	0.76 - 0.95	0.93	<0.0001	0.88 - 0.97
15	20	0.58	<0.01	0.28 - 0.84	0.89	<0.0001	0.75 - 0.95

16	20	0.73	<0.001	0.47 - 0.89	0.87	<0.0001	0.78 - 0.94
17	20	0.74	<0.001	0.49 - 0.89	0.80	<0.0001	0.62 - 0.92
18	20	0.88	<0.0001	0.77 - 0.95	0.87	<0.0001	0.67 - 0.95

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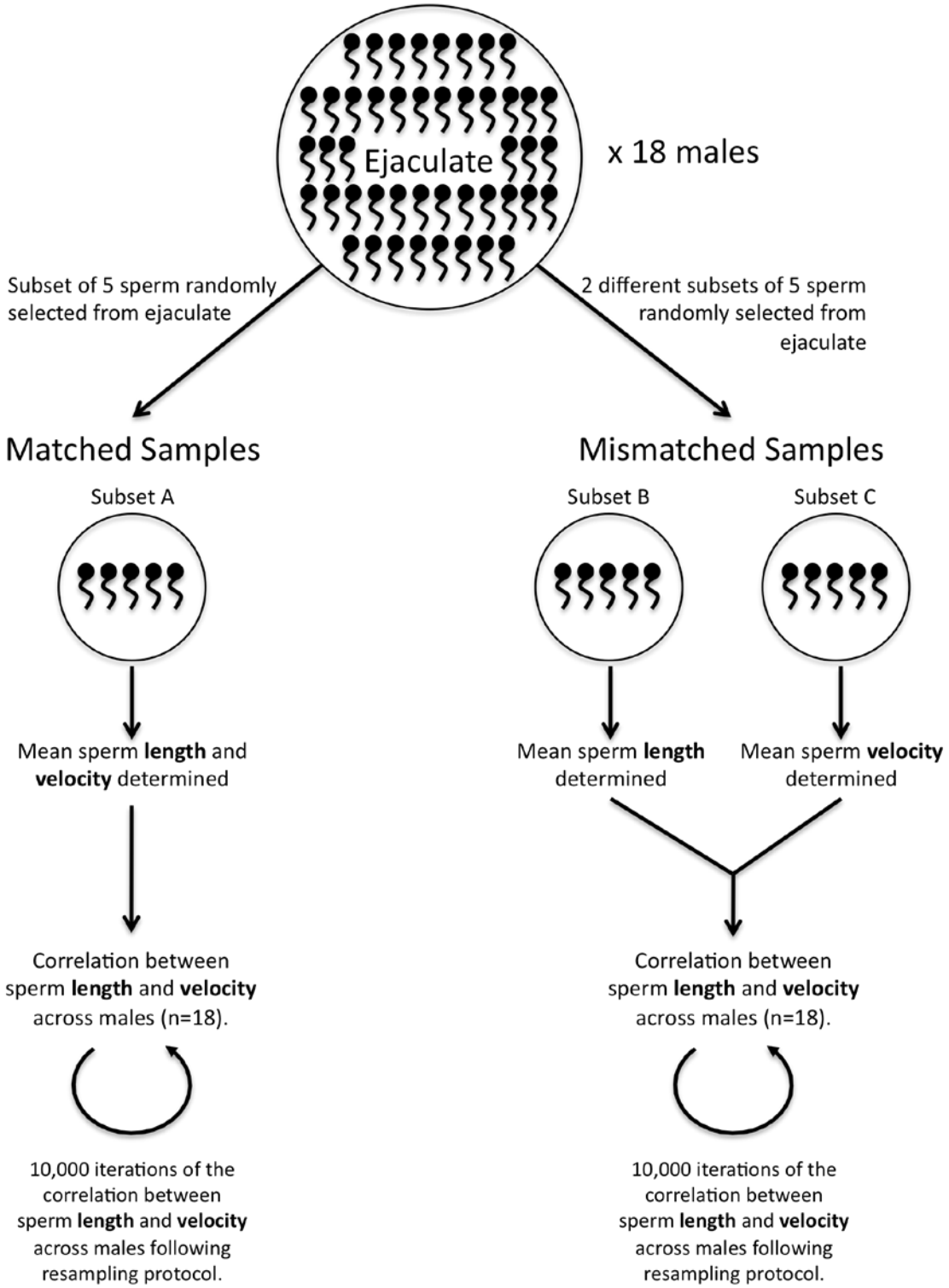
ESM Figure S1: Schematic overview of the resampling approach used to explore how intra-male variation influence sperm length-velocity relationships. We investigated sperm length-velocity relationships *among* males using sperm measurements from either *matched* (sperm length-velocity obtained from the same subset of sperm) or *mismatched* (sperm length-velocity obtained from different subsets of sperm) from the same ejaculate. The resampling approach consisted of randomly selecting subsets of sperm during every iteration of the protocol, followed by the calculation of the correlation coefficient across males. If within-male variation is negligible, covariation between sperm length and velocity following matched or mismatched protocols should be comparable, and consequently the sperm length-velocity relationship across males should not differ either under both protocols. Conversely, differences between the two protocols, matched and mismatched, will reveal substantial within-male variation affecting sperm length-velocity correlations.

ESM Figure 1


Ejaculate
x 18 males
Subset of 5 sperm randomly selected from ejaculate
2 different subsets of 5 sperm randomly selected from ejaculate
Matched Samples
Mismatched Samples
Subset A
Subset B
Subset C
Mean sperm length and velocity determined
Mean sperm length determined
Mean sperm velocity determined
Correlation between sperm length and velocity across males (n=18).
Correlation between sperm length and velocity across males (n=18).
10,000 iterations of the correlation between sperm length and velocity across males following resampling protocol.
10,000 iterations of the correlation between sperm length and velocity across males following resampling protocol.