Tactic-specific differences in seminal fluid influence sperm performance

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SUPPLEMENTARY DATA

Preliminary measurement of sperm velocity without any seminal fluid

On all individuals, employed in both experiments (with and without seminal fluid mixing), sperm velocity was preliminarily measured also after incubation in activating solution (filtered sea water at 20 °C ± 1 °C containing 2 mg/mL of bovine serum albumin), without any seminal fluid, and results were then compared with sperm velocity after incubation in each male’s own seminal fluid. This allowed to evaluate sperm velocity in the absence of any seminal fluid and to verify the general positive effect of males’ own seminal fluid. Indeed, sperm velocity significantly increased in the presence of own fluid on both territorial and sneaker males (ANOVA for repeated measures: experiment 1: tactic $F_{1,38} = 0.66$, $p = 0.42$; treatment $F_{1,38} = 51.73$, $p < 0.0001$; tactic x treatment $F_{1,38} = 1.03$, $p = 0.32$; experiment 2: tactic $F_{1,46} = 0.344$, $p = 0.56$; treatment $F_{1,46} = 93.30$, $p < 0.0001$; tactic x treatment $F_{1,46} = 10.94$, $p = 0.002$; paired t-test: all adjusted $p < 0.001$), while no differences between tactics were detected (t-test: all $p > 0.17$).

Control test on territorial male’s fluid dilution effect

In the second experiment, with seminal fluid mixing, sperm velocity of each territorial male was measured after incubation in their own seminal fluid or in a 1:1 mixture of own seminal fluid and the diluted (1:10) fluid of a sneaker male, and results proved a detrimental effect of sneaker’s seminal fluid on territorial male’s sperm velocity (see original manuscript). To exclude that this effect could be due to a dilution of territorial male’s seminal fluid, that in the treatment with mixed fluids represents half of the volume supplied in the treatment with own fluid, we also checked sperm velocity of territorial male when incubated with a diluted solution of own fluid. Thus, on a sample of 14 territorial males we measured sperm velocity after incubation with i) own fluid, ii) 1:
1 mixture of its own seminal fluid and activating solution (filtered sea water), iii) 1:1 mixture of its 
own seminal fluid and the diluted (1:10) fluid of a sneaker male.

Results proved that the detrimental effect on territorial male’s sperm velocity cannot be ascribed to 
a dilution of its own fluid but only to the presence of the rival sneaker fluid (ANOVA for repeated 
measures: $F_{2,26} = 12.72, p < 0.001$). Indeed, we did not find any significant difference in sperm 
velocity between incubation with own fluid or with a mixture 1:1 of own fluid and activating 
solution (paired t-test: $t= 1.98, p = 0.07$, d.f. 13. Fig. S1), while we confirmed that territorial males’ 
sperm were slowed by the addition of a sneaker's fluid (paired t-test: $t= 3.62, p < 0.01$, d.f. 13;
adjusted $p = 0.0062$. Fig. S1).

Fig. S1 Velocity of territorial male sperm after incubation with its own fluid, in a mix of own fluid 
+ activating solution (filtered sea water) and in a mix of own + other sneaker fluid (diluted 1:10). *** 
p<0.01