Supplemental Material Post-liquefaction normospermic human semen behaves as a weak-gel viscoelastic fluid

Giovanna Tomaiuolo,^{1, 2} Fiammetta Fellico,^{1, 2} Valentina Preziosi,^{1, 2} Federica Cariati,³ Ida Strina,³ Carmela Votino,⁴ Fulvio Zullo,³ Salvatore Longobardi,⁵ and Stefano Guido^{1, 2}

¹Department of Chemical, Materials and Production Engineering, University of Naples Federico II, Napoli, Italy ²CEINGE, Advanced Biotechnologies, Napoli, Italy

³Department of Neuroscience, Reproductive Science and Odontostomatology, University of Naples Federico II, Napoli, Italy ⁴Fetal Medicine Unit, Di Venere and Sarcone Hospitals, ASL BA, Bari, Italy

⁵Clinical Development Department, Merck KgaA, Darmstadt, Germany

In the main manuscript, we have presented a linear viscoelasticity analysis of normospermic human semen samples to assess their rheological behavior. Furthermore, we have correlated the so obtained rheological parameters to clinical data of sperm motility.

In this supplemental material, we provide additional graphs on rheological measurements and on the correlation between spermatozoa motility and rheological parameters at different values of shear rate and angular frequency.



Figure S1: The scaled elastic \tilde{G}' and viscous \tilde{G}'' modulus vs the scaled frequency $\tilde{\omega}$. The horizontal shift factor a is calculated as the reciprocal of the cross-over frequency divided by the high shear viscosity μ , while the vertical shift factor b is calculated as the reciprocal of the \tilde{G}' value corresponding to the plateau observed in the low frequency range. The inset shows the plot of the vertical shift factor b vs the horizontal shift factor a divided by the high shear viscosity μ .



Figure S2: Data of the scaled viscosity $\tilde{\eta}$ vs the scaled shear rate $\tilde{\gamma}$ for the individual samples tested in this work (a) and after scaling (b). The continuous line in (b) is a fit of the power-law equation to the experimental data at high shear rates, with exponent equal to -0.09, close to n-1. The inset shows the plot of the vertical b'' vs the horizontal a'' shift factor.



Figure S3: Data of viscosity vs shear rate for two representative samples (a-b). Closed circles represent data from low to high shear rate, while open circles represent data from high to low shear rate.



Figure S4: Plots of spermatozoa motility vs viscosity at a shear rate of (a) 1 s⁻¹, (b) 100 s⁻¹ and (c) 1000 s⁻¹.



Figure S5: Plots of spermatozoa motility vs (a) elastic modulus G' and (b) loss modulus G'' at 1 rad/s.



Figure S6: Plots of spermatozoa motility vs (a) elastic modulus G' and (b) loss modulus G' at 30 rad/s.



Figure S7: Plots of all motile spermatozoa (i.e. progressive plus *in situ* motile spermatozoa) vs (a) elastic modulus G' and (b) loss modulus G'' at 10 rad/s.