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**The effect of viscosity on spermatozoa motility and motion behaviours.**

Reproductive strategies and sperm motility are crucial determinants of successful fertilisation across species. The spermatozoa have evolved to adapt to the specific reproductive micro-environments where various factors such as temperature, osmolarity, ionic concentration, pH, and viscosity among others influence sperm motility and behaviours. Viscosity plays a pivotal role in modulating sperm movement. It affects the physical resistance encountered by spermatozoa as they swim, thereby influencing their speed, trajectory, and energy expenditure.

Even though in natural environments, viscosity from the reproductive fluids (RF) such as semen, ovarian or coelomic fluid varies significantly depending on the reproductive strategy of the species and the mode of fertilisation, studies have established that in internal fertilising species, viscosity plays a role on how sperm swim to meet and fertilise the egg. However, in external fertilising species, gametes fuse in water where the viscous RF is present, and its viscosity is higher than that of water. Nonetheless, how the presence of viscous nature influences sperm motility in external fertilisers is still largely unknown.

Therefore, we plan to investigate how viscosity impacts sperm motility from external fertilising species with different sperm sizes and shapes of various modes of fertilisation. To achieve this, the proposed study will make the species activation media viscous, using methylcellulose/ polyvinylpyrrolidone at varying viscosities. This will result in artificial viscous activation media (AVM) simulating natural viscous nature fertilisation environments. Sperm samples will be collected from the natural breeding season of species. Then motility parameters in AVM will be video-recorded using a digital camera coupled with a negative phase-contrast microscope.

Additionally, microcapillary experiments will be employed to observe sperm behaviour when transitioning across viscosity boundaries hence shedding light on the hydrodynamic challenges sperm encounter in heterogeneous environments. Video records will be analysed using the CASA (Computer-Aided Sperm Analysis) plugin for ImageJ and the data will be statistically analysed. The findings will be critical for deciphering species-specific viscous adaptations which could contribute to optimising artificial reproduction protocols and enhancing our understanding of the relationship between sperm morphology, motility, and environmental factors i.e viscosity.