

Microfluidic-Based Analysis and Selection of Mammalian Spermatozoa

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Primary CNF Tools Used: ABM Mask Aligner

Abstract:

Successful fertilization depends on sperm navigating the complex, dynamic female reproductive tract shaped by geometry and fluid flow. We used microfluidic platforms to study bovine sperm migration under two levels of structural and hydrodynamic complexity. First, we examined rheotactic swimming through tapered microchannel strictures, finding narrower angles (45°) enhance upstream progression while wider angles (90°) impede it. Second, modeling and experiments revealed a novel behavior, rotary rheotaxis, where sperm follow stable, curved upstream paths in outward radial flow. We harnessed this in a microfluidic device combining radial flow and strictures to isolate highly motile sperm from raw semen, advancing sperm–fluid–structure interaction understanding and assisted reproduction technologies.

Summary of Research:

Spermatozoa transverse through and interact with the female reproductive tract (FRT) where they are exposed to a complex microenvironment on their way to the oocyte. Rheotaxis is the reorientation of sperm in a shear flow. We used two level of flow and geometrical complexity on rheotactic sperm navigation: obstructed pathways and radial flows. The microenvironments are fabricated by manufacturing microfluidic devices using conventional soft lithography technique. We used bovine sperm as a model for mammalian sperm.

During their journey in the female reproductive tract (FRT), sperm interact hydrodynamically with complex microstructures formed by the epithelial surfaces of the fallopian tubes. Spatial heterogeneity and muscle contractions cause spatiotemporal geometry changes. Although several studies simulate the FRT using simple

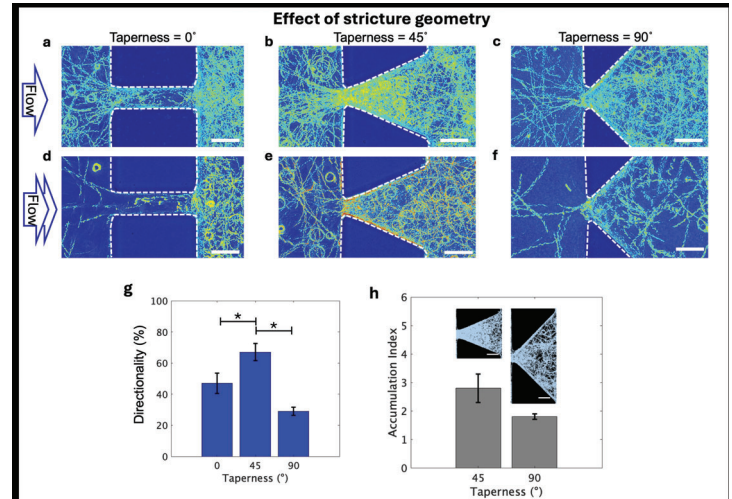


Figure 1: Obstructed pathways mimicking the female reproductive tract, showing sperm trajectories in straight barriers (a, d), tapered barriers with $\beta = 45^\circ$ (b, e) and $\beta = 90^\circ$ (c, f) under average shear rates of 8 and 12 s^{-1} . Trajectories are colorized by grayscale intensity. Scale bar: $100 \mu\text{m}$. (g) Average directionality across three barrier types under identical flow ($p < 0.0001$, one-way ANOVA). (h) Accumulation index decreases with increased taper angle from $\beta = 45^\circ$ to $\beta = 90^\circ$. Overlaid images represent 5-second sperm motion; blue pixels mark sperm presence.

sperm passage channels, none clarify how geometry alterations affect upstream sperm navigation. Previous work showed gate-like behavior of strictures in sperm migration. We systematically studied how barrier geometry affects sperm navigation, mimicking fallopian tube structure (Fig. 1(a–f)). We found that 45° tapered barriers increased navigation by 20% (Fig. 1(g)), while increasing taper to 90° obstructed sperm passage. As shown in Fig. 1(h), accumulation at the barrier apex decreases as taper increases from 45° to 90° under the same shear rate.

We observed that sperm interact and cooperate while passing through the barrier port, forming train-like groups (Fig. 2(a)). This cooperation occurs in three phases: (1) Initial Alignment: temporary orientation at the port entrance due to tapered geometry; (2) Cooperative Train Formation: flagellar synchronization in the high-shear port enhances swimming efficiency; and (3) Dispersal: group disbands upon exiting into the upstream reservoir as shear decreases. To quantify this behavior, we measured inter-sperm distances (Δr) and

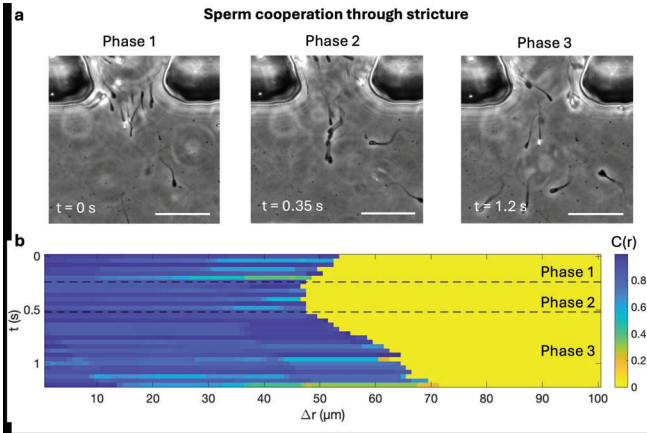


Figure 2: Sperm cooperation in the $\beta = 45^\circ$ barrier: (a) Stages: Phase 1—alignment by taper, Phase 2—cooperative train through high-shear port, Phase 3—dispersal upstream. (b) Heatmap of correlation function $C(r)$ over distance and time, showing phase transitions. Color bar: correlation values.

orientation differences ($\Delta\theta$) during a six-sperm event. The correlation function $C(r)$, calculated across distance and time, captures the spatiotemporal dynamics of sperm cooperation (Fig. 2(b)).

We demonstrated that sperm navigate outward radial flow gradients (Fig. 3(a), (b)), showing distinct rheotactic behaviors based on flow rate. At moderate rates, sperm migrated toward the center; at higher rates, they exhibited a rotary rheotaxis, spiraling inward (Fig. 3(c)). This inspired the design of the SUN chip, which combines controlled radial flow with geometric strictures to enhance sperm selection (Fig. 3(e)). Stricture geometry, based on prior work, maximizes upstream navigation while maintaining high shear rates suitable for selecting motile sperm (Fig. 3(f)). Experiments confirmed effective navigation and radial

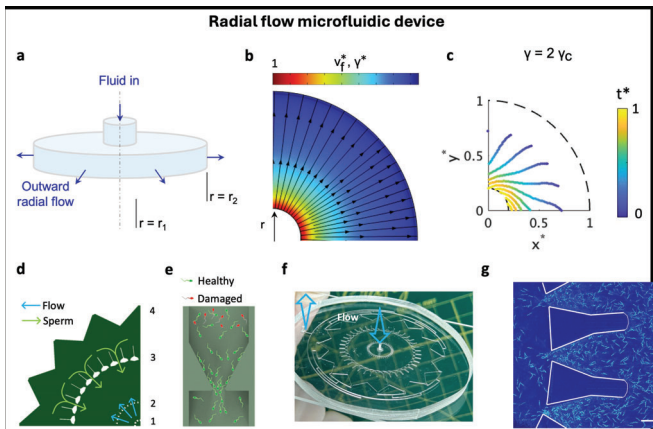


Figure 3: SUN chip design. (a) Radial flow generated by pressure difference between inner (r_1) and outer (r_2) radii. (b) Normalized velocity (v^*) decays with r ; streamlines/arrows indicate flow direction. (c) Sperm trajectories at shear rate $\gamma = 2\gamma_c$. Time and dimensions normalized for clarity. (d) Schematic of one-quarter of the chip showing flow direction and regions I–IV. (e) Sperm migration through a stricture. (f) SUN chip image. (g) Sperm navigation in region II.

flow directing sperm toward the origin (Fig. 3(g)). The SUN chip processes raw bull semen at various flow rates, isolating highly motile (>95%) and viable sperm (Fig. 4(a)). Selected sperm showed ~50% higher average path velocity (VAP) than raw semen (Fig. 4(b)). Amplitude of head oscillation (ALH) and beat cross frequency (BCF) increased significantly at most flow rates, indicating more progressive sperm behavior under low to moderate flow (Fig. 4(c)).

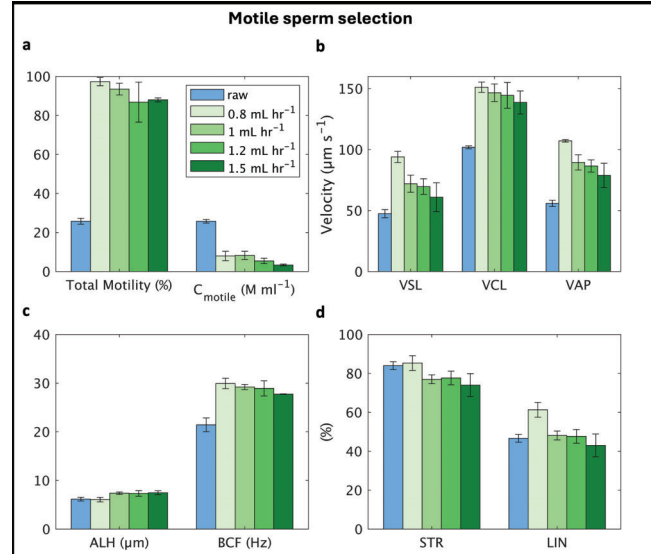


Figure 4: Motility profiles of sperm extracted via SUN chip under flow rates 0.8–1.5 mL h^{-1} vs. raw bovine semen. (a) Total motility and motile sperm concentration. (b) VSL, VCL, VAP. (c) ALH and BCF. (d) STR and LIN. SUN chip-processed samples show enhanced motility over raw semen.

Conclusions and Future Steps:

We developed microfluidic devices to study sperm migration in complex environments, focusing on obstructed pathways and radial flow. Our results show that pathway geometry, especially constriction angles, significantly influences rheotactic sperm behavior. Combining geometric strictures with radial flow, we created the SUN chip, which processes 150 μL . This work enhances understanding of microswimmer navigation in dynamic environments and lays groundwork for studying other motile microorganisms. Future efforts should scale processing volume and add automation for clinical use, particularly in IVF. The SUN chip's larger features ease fabrication for mass production, though automation and commercialization challenges remain.

References:

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