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# Modelling a tethered mammalian sperm cell undergoing hyperactivation

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## HIGHLIGHTS

- A theoretical study of an epithelially bound sperm and its flagellum is conducted.
- A hyperactivated flagellum exerts more force and can pull bound sperm away from surfaces.
- The pulling motion is regulated by flagellar beat asymmetry, wavenumber and amplitude.
- The absence of hyperactivation entails bound sperm do not pull away from surfaces.
- A favourable comparison of theoretical results and initial experiments is observed.

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## ABSTRACT

The beat patterns of mammalian sperm flagella can be categorised into two different types. The first involves symmetric waves propagating down the flagellum with a net linear propulsion of the sperm cell. The second, hyperactive, waveform is classified by vigorous asymmetric waves of higher amplitude, lower wavenumber and frequency propagating down the flagellum resulting in highly curved trajectories. The latter beat pattern is part of the capacitation process whereby sperm prepare for the prospective penetration of the zona pellucida and fusion with the egg. Hyperactivation is often observed to initiate as sperm escape from epithelial and ciliary bindings formed within the isthmic regions of the female oviducts, leading to a conjecture in the literature that this waveform is mechanically important for sperm escape. Hence, we explore the mechanical effects of hyperactivation on a tethered sperm, focussing on a Newtonian fluid. Using a resistive force theory model we demonstrate that hyperactivation can indeed generate forces that pull the sperm away from a tethering point and consequently a hyperactivated sperm cell bound to an epithelial surface need not always be pushed by its flagellum. More generally, directions of the forces generated by tethered flagella are insensitive to reductions in beat frequency and the detailed flagellar responses depend on the nature of the binding at the tethering point. Furthermore, waveform asymmetry and amplitude increases enhance the tendency for a tethered flagellum to start tugging on its binding. The same is generally predicted to be true for reductions in the wavenumber of the flagellum beat, but not universally so, emphasising the dynamical complexity of flagellar force generation. Finally, qualitative observations drawn from experimental data of human sperm bound to excised female reproductive tract are also presented and are found to be consistent with the theoretical predictions.



## 1. Introduction

Flagella are ubiquitous and slender organelles responsible for inducing fluid mixing and cell propagation in numerous settings including reproduction, filter feeding and protist pathogenicity (Lighthill, 1976). The flagellum axoneme consists of nine microtubule doublets surrounding a central pair of single microtubules (Fawcett, 1970). This phylogenetically conserved '9+2' canonical axoneme drives flagellar motility via the contraction of dynein molecular motors linking between the doublets (Vernon and Woolley, 2002). In the context of mammalian reproduction, flagellated motility is critical for the transport of sperm (Jahn and Votta, 1972), which must traverse immunologically hostile and biophysically diverse microenvironments on the journey to the egg.

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A key aspect of mammalian sperm motility is the nature of the flagellum beat. Under the microscope, this beat pattern is often characterised in terms of two distinct motility behaviours, the first being *symmetric beating*, involving symmetric waves propagating down the flagellum resulting in a net linear propulsion of the sperm (Suarez et al., 1991). In contrast, *hyperactive beating* is characterised in terms of asymmetric flagellum kinematics with increased wave amplitudes (Philips, 1972), though global and precise definitions are lacking (Ho and Suarez, 2001). Furthermore, the ability to hyperactivate is observed to be critical for fertility (Quill et al., 2003) and is part of the capacitation process, which also includes sperm head changes via the shedding of proteins and cholesterol from its plasma membrane in preparation for the subsequent penetration of the egg layers and egg fusion (Topfer-Petersen, 2002).

Calcium in particular plays a fundamental role in hyperactivation, as evidenced by the observation that extracellular calcium is required for the maintenance of hyperactivation in hamster sperm (Yanagimachi, 1994) and, given the presence of ATP and cyclic-AMP (Lindemann and Goltz, 1988), calcium initiates hyperactivation in demembranated rat flagella. Consequently, sperm calcium dynamics is critical for hyperactivation and thus fertilisation, generating the concept that disrupting CatSpers (sperm specific calcium channels Ren and Xia, 2010) may lead to a male contraception (Hildebrand et al., 2010). Thus the calcium signalling underlying hyperactivation has recently been of topical and intense interest, with investigations starting to reveal numerous aspects of the complex regulation of sperm motility. For instance, cyclic nucleotides do not appear to regulate sperm calcium via cyclic nulceotide gated channels, though they do induce calcium fluxes via CatSper channels, albeit by currently unknown mechanisms (Ren and Xia, 2010). Similarly, complex behaviours can be elicited by manipulating sperm calcium IP3 stores, such as reversing the asymmetry of the hyperactivated waveform (Chang and Suarez, 2011), though understanding the functional role of calcium stores and such behaviours in hyperactivation requires further investigation (Olson et al., 2011a).

However, despite such interest, the exact mechanism for the induction of waveform asymmetry on hyperactivation is not known, though demembranated flagellar studies do indicate the regulation occurs in the axoneme, and calcium–calmodulin complexes have been at least partially implicated (Brokaw, 1991; Ho and Suarez, 2001; Olson et al., 2011a). In contrast to understanding the complex regulatory dynamics underlying the hyperactivated waveform, its kinematical characterisation is relatively straightforward, especially for rodent studies where the falciform sperm head highlights a natural body-fixed reference frame for observations. In particular, Ohmuro and Ishijima's work on golden hamster sperm (Ohmuro and Ishijima, 2006) suggests that the hyperactivated flagellum wavenumber is reduced by 4/7th compared to symmetric beating and the frequency is reduced by around a factor of 0.25–0.35. They also show that the waveform amplitude increases by three or four for hyperactive beating relative to symmetric waveforms. These altered kinematics of the flagellar beat pattern associated with hyperactivation are observed, in golden hamsters (Stauss et al., 1995), to facilitate the successful penetration of the zona pellucida and are hypothesised to enable sperm to surmount rheological and geometrical impediments within the female reproductive tract (Suarez et al., 1991). Nonetheless, the mechanical implications of hyperactivated flagellar beat patterns are underexplored.

A specific scenario of interest concerns the binding of mammalian sperm to the epithelial surfaces within the oviductal isthmus, where the acrosomal region of the sperm head fuses to cells and cilia lining the tract walls, causing the sperm to be tethered. In mammals exhibiting oestrous, the sperm are seen to avidly bind within these regions until ovulation, where only a few manage to escape at a time; this is hypothesised to reduce the likelihood of polyspermic fertilisation (Suarez, 2007). Another postulated function of this 'oviductal reservoir' is to prolong the viability of the sperm and regulate the capacitation process. Observations suggest that the release of a sperm cell from a binding site is induced by changes brought about by the sperm itself as opposed to being a result of external changes in the epithelial wall when close to ovulation, although epithelial signalling could still be a possibility (Suarez, 2007).

Given that the flagellum is always observed to push the sperm cell body during swimming, one may anticipate that flagellar motility will not contribute to bond breaking as it would thrust the cell further into the epithelium. In contrast, the escape of sperm does indeed appear to be correlated to capacitation and the induction of hyperactivation (Demott and Suarez, 1992), leading to the hypothesis that flagellar mechanics are in fact important in sperm release. Thus our objective in the following study is to explore whether it is mechanically feasible for hyperactivation to assist in the epithelial escape of mammalian sperm, at least in the context of Newtonian fluid dynamics.

There are numerous formalisms for studying flagellar mechanics, ranging from resistive force theory (Lighthill, 1976; Gray and Hancock, 1955) to direct numerical solutions via boundary elements (Shum et al., 2010) or immersed boundaries (Dillon et al., 2007), with a spectrum of accuracy which is inversely related to the ease of implementation, as reviewed by Gaffney et al. (2011). More recently, the method of regularised Stokeslets (Cortez, 2002) has also been used to explore the motion of sperm cells, for instance in the investigation of hyperactivity coupled with calcium dynamics (Olson et al., 2011b). Here, we use resistive force theory (Lighthill, 1976; Gray and Hancock, 1955), a classical means of modelling the connection between flagellar waveforms and the viscous drags associated with inertialess Newtonian fluid dynamics. This modelling framework is straightforward to implement but is only a leading order expression for the relationship between the viscous drag and velocity of a slender body element (Lighthill, 1976; Johnson, 1980), which is typically considered in the absence of nearby surfaces. Nonetheless, this approach gives reasonable agreements with observations of motile bull sperm swimming near to a surface (Friedrich et al., 2010). This is due, in part, to the extreme slenderness of a flagellum and the fact that the flagellum has to be very close to a surface for the resistive force theory relationship between viscous drag and velocity to be violated, especially in the context of the current study as further illustrated in Section 3. An additional mechanism for the breakdown of resistive force theory arises due to flows induced by the cell body (Johnson and Brokaw, 1979). However, this is not relevant in the current context, as mammalian sperm heads are sufficiently small so as not to endanger resistive force theory in this manner (Johnson and Brokaw, 1979) and, here, the sperm head is highly constrained due to tethering which further reduces the flow field it generates. Consequently, resistive force theory provides a simple means of generating leading order estimates for the forces and torques exerted at a sperm's tethering point, as we further document in developing the modelling framework and exploring its predictions below.

In the following section, we summarise the resistive force theory modelling formalism and we also detail an experimental methodology for observing human sperm bound to an epithelial substrate. This is followed by a description of the modelling predictions for the flagellar forces exerted by symmetric and hyperactive waveforms, which are contrasted for sperm that are attached to a substrate via a torque free hinge and via a clamping that does not allow head rotation. We also consider a qualitative comparison of modelling predictions and observation, before detailing our conclusions in the final discussion section.