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NEW RESEARCH HORIZON ***Review***

Sperm motility: is viscosity fundamental to progress?

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ABSTRACT: The success of internal fertilization is reliant upon successful sperm migration through the female tract. Timely location of the oocyte in what is a complex three-dimensional, highly invaginated series of moist opposed surfaces is a challenge at which only tens of sperm ever succeed. In part this could be due to the differences in scale, with a 50 μm long cell facing a probable migration of well over 20 cm due to the complex architecture. Many groups have focused upon the role for a chemotactic 'attractive egg' effect in guiding sperm to increase numbers at the fertilization site. What most research has neglected to consider is the role that the viscosity of the mucous layers, which coat the entire tract and through which sperm must swim, plays in both sperm selection and ongoing modulation of their behaviour. From allowing sperm to enter through the cervix during the ovulation phase, to denying them entrance through action of the female contraceptive pill, viscous effects are fundamental in controlling the migrating sperm population throughout the tract. The physiological effects of viscosity are also crucial to consider when designing and extrapolating data from *in vitro* experiments to the *in vivo* situation.

Key words: viscosity / cervical mucus / oviductal secretions / sperm motility / flagellum

Introduction

Since the pioneering work of Kremer (1965, 1976) it has been known that migration of sperm through the viscoelastic cervical mucus is a useful indicator of natural fertility, with workers having carefully imaged sperm migration and noted motility and migration differences from in standard laboratory media or those used in assisted reproduction technologies (ART), which are low viscosity (e.g. Katz *et al.*, 1978). More recently the concentration of sperm migrating a set distance through mucous or viscous analogues has been identified as a more accurate predictor of fertility than the previous-used measure of 'vanguard distance', i.e. the maximum migration distance (see systematic review by Ola *et al.*, 2003, also earlier papers by Mills and Katz, 1978; Mortimer *et al.*, 1990; Aitken *et al.*, 1992). This has also been successfully incorporated into an over-the-counter sperm test (Björndahl *et al.*, 2006). We hypothesize that the reason the standard semen analysis assessment of rapidly progressive sperm in raw ejaculate is a useful indicator of fertility (Björndahl 2010), is that they are demonstrating the ability to migrate well in a viscous medium: semen.

Immediately upon semen deposition in the cervix, sperm start to migrate into the mucus; potentially sperm could even enter mucus without being mixed with the seminal vesicular secretions associated with ejaculated clotting. If this mixing does not occur there may then be implications for the changes in chromatin structure affected by zinc (reviewed in Björndahl and Kvist, 2010). This is the beginning of a migration process that will essentially involve them crawling in mucous layers, of differing viscosities, until they reach the oocyte or, much more likely, die. Little is known about the viscosities present further along the sperm journey, the most detailed data available those of Jansen (1980), though these are far from comprehensive. As cilia are present throughout the female tract the specific requirements for sperm to avoid them at this site, or indeed anywhere within the female tract, remains unclear.

The viscous contrast between media and mucus

To better understand how viscosity can influence a sperm, we need to consider mathematical fluid mechanics. The fluid mechanics of the male gamete has recently been reviewed in detail (Gaffney *et al.*, 2011), so here we choose to summarize the two key points.

Viscosity dominates, even in saline

Our intuitions about swimming and propulsion through water may be misleading when thinking about sperm. A rapidly motile sperm viewed under the microscope appears somewhat like a small boat with a very large and powerful outboard motor, thrashing through the fluid. However, the fluid flow around the cell is completely different from what might be expected. A motor boat produces a turbulent wake, whereas a sperm produces no wake at all (Fig. 1). The reason for

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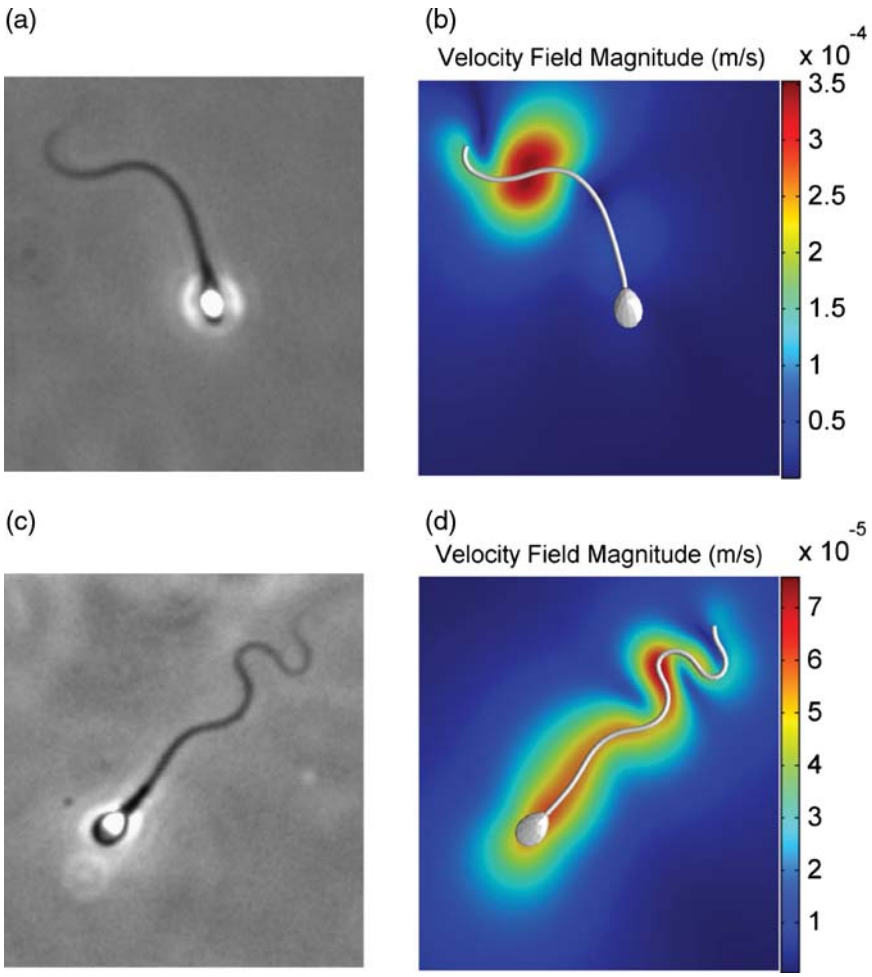

Figure 1 Imaging and associated fluid mechanics simulation of sperm swimming. (**a** and **b**) Show a migratory human sperm in standard viscosity medium; (**c** and **d**) show a migratory human sperm in medium with viscosity similar to ovulatory human cervical mucus, the meandering pattern in physiological viscosity being evident. The colour plots in (b and d) show the velocity field magnitude in a plane a distance 2.5 μm below the cell, showing the absence of any wake or turbulence. Both cells have similar progressive velocity (corresponding to the CASA measure of VSL). Note a different colour scale is used because the physiological viscosity cell performs much less yaw. Reprinted from Gaffney *et al.* (2011), methods given in Smith *et al.* (2009a, b). Copyright Annual Reviews, 2011.

this is that the microscopic scale means that inertia—the tendency of fluid to continue moving when disturbed—is miniscule compared with viscosity. The internal friction of fluid causes it to slow down, a phenomenon that is most familiar to us for liquids such as maple syrup, but is also present in fluids such as saline and water—for example, stirred tea eventually stops moving because of viscous energy dissipation. Mathematically, the viscous-dominated regime corresponds to a very small 'Reynolds number' and the fluid flow can be modelled and interpreted using the Stokes flow equations, which describe situations where viscosity prevails over inertia—a macroscopic example would be a person trying to swim through cold butter. These concepts were known over 60 years ago to Gray and Hancock (1955), and have since inspired the famous film of Taylor (1967), and Purcell's lecture *Life at Low Reynolds Number* (Purcell, 1977). A key feature, sometimes referred to as the Scallop Theorem, is that simple 'flapping' motions are not able to produce motility on these length scales. Sperm solve this problem by producing a bending wave, which propagates along the flagellum (Fig. 2); this is a common feature to all sperm, from those that migrate through seawater, to those that migrate through viscoelastic mucus.

Another feature of the viscosity-dominated regime that is not accessible to everyday intuition is the fluid dynamic effect of the fluid friction at surfaces, such as microscope slides, coverslips and the oviductal epithelium. This results in the well-known phenomenon of surface accumulation, first reported quantitatively by Rothschild (1963), and investigated experimentally (Woolley and Vernon, 2001; Cosson *et al.*, 2003) and computationally (Fauci and McDonald, 1995; Smith *et al.*, 2009a, b). Fluid dynamic effects may cause sperm to swim both near and against surfaces, depending on their flagellar movement, and species-specific features of head morphology and head/flagellum relative size (Lazebnik, 2002; Smith and Blake, 2009; Smith *et al.*, 2011).

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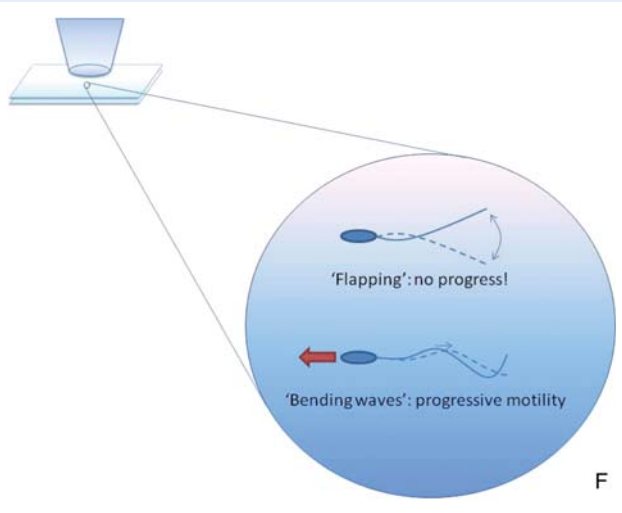

Figure 2 On the microscale, sperm are subject to a very different balance of physical effects from macroscopic swimmers. In particular, time-reversible 'flapping' motions would not successfully propel the cell, hence sperm generally exhibit bending wave propagation, which have an inherent directionality.

Mucus is not saline

As explained above, viscous forces dominate the motion of sperm in all fluids, from mucus (Fig. 1c and d) to saline (Fig. 1a and b). However, the greatly increased viscosity of mucus relative to saline imposes greatly increased resistance to progression, resulting in a very different 'meandering' waveform in mammalian cells and reduced head yaw (side-to-side movement across the directional axis; Fig. 1c and d). While mucus is a complex fluid that does not have a clearly defined viscosity, a Maxwell fitting procedure suggests an effective viscosity of mid-cycle mucus being ~200 times that of saline (Smith *et al.*, 2009b, re-analysing the data of Wolf *et al.*, 1977). Despite this greatly increased resistance, human sperm are able to migrate with similar progressive velocity in physiological viscosity fluid compared with saline. Externally fertilizing sperm are not adapted to maintain progressive velocity in high-viscosity fluids (Woolley 2003). Finally, the increased viscosity results in greatly increased metabolic requirements, which may be satisfied by glycolysis (Ford 2006; Storey, 2008). Data from our group suggest that the characteristic waveform of mammalian high-viscosity swimming may provide significant improvements in efficiency; nevertheless, this factor must be taken into account when comparing external and internal fertilization. The change in swimming trajectory and head yaw also emphasize that it is not straightforward to extrapolate motility changes in saline, for example due to chemotaxis, to a physiological function. Moreover, a recent theoretical and computational study (Gadêlha *et al.*, 2010) suggests that increased viscosity may cause the sperm flagellum to undergo a 'buckling instability' that results in asymmetric flagellar beating and circling motility (a form of 'trapping'), without any need for asymmetric internal actuation. We have observed this type of high viscosity 'trapping' in high-viscosity migration experiments (reported and interpreted mechanically in Gadêlha *et al.*, 2010).

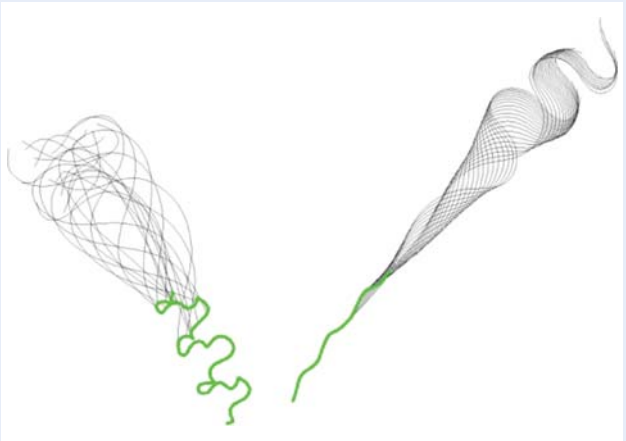

Figure 3 Plots of sperm in standard low-viscosity medium (left) and ovulatory physiological viscosity medium (right), showing a time-lapse of flagellar positions (black lines) and the trajectory of the cell (green lines). Yaw (side-to-side movement across the directional axis) is greatly reduced at high viscosity; progressive velocity is similar despite the greatly increased viscous resistance to movement. Reprinted from Smith *et al.* (2009b), copyright Wiley.

In vitro observations of viscous effects upon sperm

David Woolley and colleagues in an innovative series of experiments with hamster and mouse sperm developed many of the techniques and told us much about the flagellar beat as we understand it today (Woolley, 1977, 1981; Cooper and Woolley, 1982; Yeung and Woolley, 1983, 1984).

The first detailed study of sperm in high-viscosity fluids appears to be that of Brokaw (1966), the beginning of a programme of experimental and theoretical research investigating the coupling of axoneme and viscous drag by modulating the viscous drag (for other examples of work and commentary on this major research effort, see for example Brokaw, 1975; Pate and Brokaw, 1980; Rikmenspoel, 1984; Brokaw, 2002; Pelle *et al.*, 2009; Woolley *et al.*, 2009; Lindemann, 2010; Woolley, 2010). While a handful of studies have examined the human sperm waveform in high-viscosity fluids (Katz *et al.*, 1978; Ishijima, 1986; Smith *et al.*, 2009b and Fig. 3) we are not aware of significant findings linking waveform modulation and migrational 'guidance', in high viscosity, as would be required in a model of physiological chemotaxis.

In vitro: how far to go?

Independently of research considering realistic scale and fluid mechanic effects on human sperm, research into different selective and tactic responses of the sperm has developed. These include suggestions such as thermotaxis (Bahat *et al.*, 2003, 2006, 2010); more detailed observations from many laboratories of human sperm chemotaxis (to odorant molecules led by Spehr *et al.*, 2003, 2004, 2006, and progesterone at picomolar levels by Laura Giojalas, e.g. Teves *et al.*, 2006, 2009); and stimulus-specific cell behaviours evoked via different chemoreceptors by Veitinger *et al.*, 2011. All of these studies have been undertaken in conventional laboratory ART media at low viscosities; this may mean

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the data are confounded if, as a consequence of the treatments, hyperactivation occurred. In the post-cervical stages of migration and fertilization the viscosity terrain of the uterus and fallopian tubes will in general consist of fluids of higher viscosity than ART media; data on human female tract viscosity are limited but observations suggest that viscous mucus may appear transiently in the isthmus (Jansen, 1980), moreover the hyaluronate gel matrix of the cumulus is a highly viscoelastic semi-solid. This is important because viscosity will affect flagellar movement and cell trajectory (Gadelha *et al*., 2010) and because chemotactic cell accumulation observed experimentally may be a result of hyperactivation, a low-viscosity cell behaviour.

The phenomenon of 'hyperactivation', observed as a wild thrashing motion in standard salines, is indicative of an out of control increase in yaw of the sperm head, due to lack of viscous damping of active flagellar bending. At physiological viscosity these sperm would swim very effectively forwards as the viscous forces would 'eliminate' the yaw, as indicated by data in mouse sperm (Suarez and Dai, 1992). Hence, previous observations of sperm trapping due to hyperactivation, at saline viscosity (e.g. Jaiswal *et al*., 1999) need re-interpretation or repeating in light of outcomes in physiological viscosity.

Undertaking chemotaxis experiments in a physiological viscosity is technically difficult because viscosity will also affect formation of a gradient; the classical Einstein diffusion theory implying that a 200-fold increase in viscosity will increase diffusion coefficient, and hence a 200-fold increase in time taken to establish a gradient. The effect of this speed of gradient formation, versus mixing by surface cilia, the passage of sperm and the likely irregular 'stirring' smooth muscle contractions (Vizza *et al*., 1991) in the human fallopian tube remain to be elucidated. Any gradient of fluid from the ovulated cumulus oocyte complex would therefore be intricate in structure and its form is totally unknown. Follicular progesterone diffusion from the wall of the fallopian tubes as part of a counter-current exchange system has been demonstrated to modulate sperm binding (reviewed in Hunter 2008), it is possible this system may also set-up gradients in the tube. Various workers have concentrated upon the role of how the biochemical constituents of the oviduct can affect gametes (reviewed by Aviles *et al*., 2010); these data now need exploiting fully to unravel the metabolic and regulatory needs of a sperm in a realistic viscosity, thereby highlighting potential fertility and contraceptive targets.

The effects of the complex internal folded ciliated epithelium on sperm movement have yet to be fully understood; data suggest that ampullary sperm may perform a 'bind and release' motility (Connolly and Kirkman-Brown, Unpublished data). Magnetic resonance imaging has started to reveal more about peri-ovulatory peristaltic contractions in the uterus (Kido *et al*., 2008). As this technology evolves further no doubt more detail of these mechanisms for sperm and liquid transport throughout the female tract will be established in detail.

Finally as sperm approach the oocyte they are confronted with the cumulus mass. Although this has been examined biochemically as a marker for embryo and pregnancy outcome (Assou *et al*., 2010) data on effects of different viscoelasticity of the structure on fertilization itself are not available.

Conclusions

When examining previous work, in light of our assertions around physiology of the tract, it is important not to 'throw the baby out with the bathwater', rather we appeal for care in designing new experiments and interpreting data in a physiological and mechanistic context from existing published work. We re-iterate the suggestion of Lazebnik (2002), in his thought provoking article 'Can a biologist fix a radio?' to the field of research across sperm motility and internal fertilization, for a 'wholeistic' approach as opposed to the traditional narrow focus on single components of signalling pathways, as beloved of us all as cell biologists.

As research tends towards examination of the 'correct cohort' of sperm that has a natural fertilizing potential, in a physiological viscosity, it is also crucial that the cells studied are prepared via selection of a population that can migrate into a viscous environment from semen. A key factor in future research will be the evolution of caged compounds, particularly caged progesterone as already used on human sperm (Kilic *et al*., 2009) and caged cyclic nucleotides that have been perfected to study invertebrate sperm (e.g. Bönigk *et al*., 2009). These should theoretically allow concentration gradients to be dynamically created in a controlled manner within viscous media, eliminating the problems with forming gradients and for the first time unlocking behaviour in a physiological media viscosity. It is tempting to speculate that the true method of human sperm chemotaxis may be via displaced circling, as for the sea urchin, and as recently observed and modelled by our group in high-viscosity mucus (Gadêlha *et al*., 2010).

In basic research, modern digital high-speed imaging alongside fluid mechanic analysis of the flagellar waveform (e.g. Smith, 2009b) provides a tangible way to study the metabolic requirements and organization of the flagellar beat, to potentially assess detailed toxicological and drug effects on sperm motility and to understand how changes to the flagellar beat can cause chemotaxis. The recent identification of progesterone action on the Catsper channel (Lishko *et al*. 2011; Strünker *et al*., 2011) and further developments and insight this will allow means knowledge in many aspects of this system is suddenly advancing together.

From assessing effects of viscosity on IUI where transition of the prepared sperm from media to the more viscous *in vivo* environment is likely to be a major hurdle; developing diagnostics that involve viscous selection; through to re-thinking how modulation of viscosity in the female tract may enhance fecundity (e.g. thinning of cervical mucus with common expectorant cough medicine, Check *et al*., 1982), a re-evaluation and awareness of viscous effects throughout the sperm journey may hold hope for new diagnoses and treatments in the field of ART.

Authors' roles

J.K.B. led the writing of this article, but both J.K.B. and D.J.S. jointly wrote and synthesised the background research.

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