Rheotaxis Guides Mammalian Sperm

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Summary

Background: In sea urchins, spermatozoan motility is altered by chemotactic peptides, giving rise to the assumption that mammalian eggs also emit chemotactic agents that guide spermatozoa through the female reproductive tract to the mature oocyte. Mammalian spermatozoa indeed undergo complex adaptations within the female (the process of capacitation) that are initiated by agents ranging from pH to progesterone, but these factors are not necessarily taxic. Currently, chemotaxis, thermotaxis, and rheotaxis have not been definitively established in mammals.

Results: Here, we show that positive rheotaxis, the ability of organisms to orient and swim against the flow of surrounding fluid, is a major taxic factor for mouse and human sperm. This flow is generated within 4 hr of sexual stimulation and coitus in female mice; prolactin-triggered oviductal fluid secretion clears the oviduct of debris, lowers viscosity, and generates the stream that guides sperm migration in the oviduct. Rheotactic movement is demonstrated in capacitated and uncapacitated spermatozoa in low- and high-viscosity media. Finally, we show that a unique sperm motion, which we quantify using the sperm head’s rolling rate, reflects sperm rotation that generates essential force for positioning the sperm in the stream. Rotation requires CatSper channels, presumably by enabling Ca2+ influx.

Conclusions: We propose that rheotaxis is a major determinant of sperm guidance over long distances in the mammalian female reproductive tract. Coitus induces fluid flow to guide sperm in the oviduct. Sperm rheotaxis requires rotational motion during CatSper channel-dependent hyperactivated motility.

Introduction

Marine invertebrate spermatozoa, such as those of sea urchin, are guided by chemotaxis; egg-secreted peptides induce changes in the arc of spermatozoan swimming paths that increase fertilization success [1]. In mammals, the route to the egg is more restricted, but it is widely assumed that follicular fluid contains chemoattractants [2]. Prompted by reports of the presence of a large number of putative odorant G protein-coupled receptors in spermatozoa, the synthetic odorants bourgeonal and lyral were proposed to mimic unknown native chemoattractants of human or mouse sperm, respectively [3, 4]. However, because muscle contraction and ciliary currents would disrupt gradients over long distances, chemotactic guidance would likely be relevant only as sperm approach the egg [5, 6]. Another hypothesis, thermotaxy, is based on ovulation-dependent small temperature gradients between the isthmus and ampulla [7].

Rheotaxis, observed in many aquatic species, is the orientation of cells and organisms within a gradient of fluid flow. Positive rheotaxis, the tendency of a cell to swim against the flow, was first reported for spermatozoa over 100 years ago [8]. While ciliary current toward the distal oviduct was observed in turtle, pigeon, and rabbit, central lumen counterflow prevented a consensus about the physiological relevance of rheotaxis, and it was concluded to be a laboratory artifact [9]. In addition to cilia-directed flow, muscle contraction, tortuous geometry, the propensity of sperm to stick to surfaces, and high-viscosity fluids were recognized as factors complicating sperm transport. Rothschild [10] noted that the sperm head is attracted to surfaces (the wall effect), and suggested that the “lighter” tail is subjected to more force in velocity gradients, resulting in the tail being dragged downstream with the head pointed upstream [11]. More recent considerations explain the wall effect as a consequence of hydrodynamics in tubes [12].

Fluid viscosity in the female reproductive tract varies, with the highest being in the cervical mucus (200 to 680 cP in humans [13]) and the oviducal isthmus [14]. Oviducal viscosity varies during the estrus cycle. Around ovulation, ciliated cells in the isthmus epithelium are covered by a dense, tenacious mucus that disappears after ovulation [14]; estrogen-regulated secretion of oviductal fluid increases 2- to 3-fold at estrus [15]. Fluid viscosity also affects sperm movement—as viscosity increases, sperm flagella propagation velocity, wave amplitude, and energy expenditure decrease [16]. Sperm angular speed (rotation around the long axis of sperm) also decreases at higher viscosities, resulting in more planar movement [17]. In the complex process of capacitation (that is, the changes that sperm undergo in the female reproductive tract that enable them to fertilize the egg), sperm develop hyperactivated motility. Hyperactivation is characterized by higher-amplitude, asymmetric waveforms of reduced beat frequency [18], which enables high flagellar swimming speeds in viscous solutions [19].

We have reinvestigated sperm taxis in vitro and in vivo using new tools that enable control of hyperactivated motility and enable the visualization of sperm movement in vivo. Here, we show that sexual intercourse in mice induces robust secretion and fluid flow within the oviduct. Sperm are directed by, and swim against, this flow (positive rheotaxis). Importantly, axial rotation requires CatSper channels for reorientation of sperm in tangential flow. We conclude that rheotaxis is a major influence in the long distance guidance of mammalian sperm to the egg.

Results

Coitus Induces Fluid Flow from Oviduct to Uterus

Oviductal epithelia actively secrete fluid into the oviductal lumen, as first reported in rabbits during estrus [20]. Uterine fluid is most abundant (91 ± 26 μl) on the day of coitus [21]; however, the origin of fluid and the mechanism of its production has not been well documented. We analyzed the accumulation of oviducal fluid in the uterus in mice. Although the uterus
Supplemental Inventory

1. Supplemental Figures and Tables
   Figure S1, Related to Figure 1
   Table S1, Related to Figure 4

2. Supplemental Movie Legends

3. Supplemental References
Figure S1, Related to Figure 1. Sperm Movement in the Ex Vivo Oviduct

(A) Cell debris/globular cells discharged from the oviduct via the isthmus (UTJ). The oviduct of a mated female mouse was removed and incubated in HTF medium for 5h. Scale bar: 200µm.

(B) Oviductal ligation near the utero-tubal junction (UTJ). Oviducts were dissected from mice 60-90 min after mating; one of the two oviducts was ligated near the UTJ in order to block fluid flow from the ampulla (white arrows) to the UTJ (red arrows). Scale bar: 1mm.

(C) Transgenic sperm in the ampulla. DsRed2 (red, tail) and EGFP (green, head)-expressing sperm in the ampulla; unligated oviduct under fluorescence microscopy. This particular spermatozoon did not undergo the acrosomal reaction (retained acrosomal EGFP).
### Table S1, Related to Figure 4. Viscosity of Luminal Fluid and Media

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<th>Viscosity (cP)</th>
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</tr>
<tr>
<td>Oviduct [2]</td>
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<td>1.8</td>
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<tr>
<td>Mouse, Uterus, 4 h after coitus</td>
<td>Total fluid</td>
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<tr>
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<td>Total fluid</td>
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<td>18,000 x g, 5 min supernatant</td>
<td>n.d.</td>
</tr>
<tr>
<td>Mouse, Uterus, 8 h after coitus</td>
<td>18,000 x g, 5 min supernatant</td>
<td>2.4 ± 0.9⁹</td>
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</table>

⁹Viscosity was greater than that of 99% glycerol at 22°C.
⁹Mean ± s.d. (n = 4); Only samples >100 µl were measured.
⁹Not determined due to low volume (see Figure 1c-ii).
⁹Mean ± s.d. (n = 3); Only samples >100 µl were measured.
⁹Not applicable.
Supplemental Movie Legends

Movie S1. Fluid Flow in the Oviduct
(A) Fluid flow in the oviductal isthmus. An oviduct removed from a mated female mouse was incubated at 37°C. Movement of cell debris/globular cells in the isthmus was observed through its transparent wall and recorded by a CCD camera at 30 fps. Left and right of the field are oriented to uterus or ovary, respectively.
(B) Ciliary current in oviductal ampulla. An oviduct dissected from a mated female mouse was mounted on a glass slide. Ciliary current in the ampulla was observed through the transparent wall and recorded by a CCD camera at 60fps. Bottom right and upper left of the field are oriented to uterus and ovary, respectively. Objective: 20x.

Movie S2. Sperm Rheotaxis In Vitro
(A) Uncapacitated mouse sperm left in the 'no-flow' condition.
(B) Uncapacitated mouse sperm in flow generated by convection (10 µm/s).
(C) Uncapacitated mouse sperm under uniform laminar flow (generated by a pipette; 12 µm/s). Sperm are incubated in M2 at 37°C. 10x objective. 60 fps.

Movie S3. Mouse Sperm Rheotaxis In Vitro
(A) Uncapacitated mouse sperm in the 'no-flow' condition in M2 medium at 37°C; 60 fps.
(B) Uncapacitated mouse sperm under fluid flow (16 µm/s) in M2 medium at 37°C; 30 fps; 4x objective.

Movie S4. Human Sperm Rheotaxis In Vitro
(A) Uncapacitated human sperm in the 'no-flow' condition.
(B) Uncapacitated human sperm under fluid flow (10 µm/s). Sperm were incubated in HEPES-HTF at 37°C; 10x objective; 60 fps.

Movie S5. Sperm Movement in a Capillary Tube during Low Viscosity Fluid Flow
(A) Loading of mouse capacitated sperm into a glass capillary tube at 210 µm/s in HEPES-HTF at 22°C. 10x objective.
(B) Immotile, aggregated, and sluggish sperm are swept out of the capillary tube by outward-directed flow at 68 µm/s at 37°C; 20x objective; 30 fps.

Movie S6. Sperm Selection in a Capillary Tube during Fluid Flow
Capacitated mouse sperm were loaded as in Movie S5A; (A) immediately after loading; (B) followed by addition of sperm-free media (HEPES-HTF) to the left end of the tube. By increasing the distance sperm had to swim, this procedure resulted in selection of active sperm. Sperm take longer reach to the end of the capillary against flow, but sluggish sperm are eliminated. 77µm/s; 37°C; 30 fps.
Movie S7. Enhanced Progressive Motility in Viscous Medium; No Flow
(A) Uncapacitated mouse sperm in HEPES-HTF (~3 min).
(B) Capacitated mouse sperm in HEPES-HTF (2 h) at 37°C; 10x objective; 60 fps. Note the transition from planar circular motion before capacitation to more linear paths during capacitation.

Movie S8. Sperm Movement in a Capillary Tube during Viscous Fluid Flow
(A) Uncapacitated mouse sperm swim along the inside surface of the capillary tube; M2 media (0.3% (w/v) MC) at 37°C; 58 μm/s; 20x objective; 30 fps.
(B) Capacitated mouse sperm swim in the center of the capillary; HEPES-HTF (0.3% MC) at 37°C; 61 μm/s; 30 fps.

Movie S9. Sperm Turning in Flowing Solution
The path of the turning sperm is marked by the dotted line. Uncapacitated mouse sperm in M2 medium at 37°C; 11 μm/s; 10x objective; 60 fps.

Movie S10. Headless Sperm Rheotax
Flow rate = 6.2 μm/s in uncapacitated conditions in M2 medium; 10x objective; motion slowed 10 fold to 6 fps.

Movie S11. Sperm Lacking CatSper Channels Have Low Motility, Swim in Circles, and Do Not Rotate at 37°C
(A) No flow; (B) flow = 15 μm/s, 4x objective; (C) flow 15 μm/s, 20x objective. Uncapacitated sperm (HEPES-HTF for 5 min only); 60 fps. Sperm dissected from the cauda epididymis of CatSper1−/− mice.

Movie S12. Lack of Rheotaxis in Sea Urchin Sperm Regardless of Fluid Flow
(A) No flow.
(B) Laminar flow 17 μm/s; 22°C. Motion slowed 2-fold to 60 fps.

Supplemental References
viscosity decreased over time (Table S1). Beginning a few seminal fluid, cell debris, and globular cells, but the fluid's ered within 4 hr after coitus was a highly viscous colloid of released cell debris and globular cells (see Figure S1 A showed that the uterine accumulation included oviduct- a significant increase in uterine fluid. Because in vitro culture at estrus required coitus; unmated control mice did not exhibit 4 hr after mating). This increase in secretion above baseline increased over time to a peak of 87
6 hours after coitus, significant secretion of bicarbonate-rich its pH from 7.1–7.3 to 7.5–7.8 [22]. Mouse uterine fluid recov-

monkeys, bicarbonate secretion into the oviduct increases during the follicular phase of the estrus cycle. During ovulation in rhesus monkeys, bicarbonate secretion into the oviduct increases its pH from 7.1–7.3 to 7.5–7.8 [22]. Mouse uterine fluid recovered within 4 hr after coitus was a highly viscous colloid of seminal fluid, cell debris, and globular cells, but the fluid’s viscosity decreased over time (Table S1). Beginning a few hours after coitus, significant secretion of bicarbonate-rich oviductal fluid increased the pH to ~8.3, reduced mucous viscosity, and washed out viscous cellular debris (Figure 1C; Movie S1A). Cilia sweep fluid and the egg toward the uterus, reducing the attachment of cells to the wall of the oviduct (Figure 1D; Movie S1B). Ampullar flow, measured 3–4 hr after coitus, was 18.0 ± 1.6 µm/s at 22°C. The increase in sperm number over the in vivo situation may result from manipulation or a change in hormone status perhaps detaching some sperm from the isthmal crypts and facilitating sperm migration. All graphs: mean ± SEM (black bars) and median with interquartile ranges (green boxes).

Genital stimulation and coitus evoke complex neurological responses. To simplify our task of determining factors that might increase oviductal flow, we considered known secreted hormones that were (1) elevated in serum after sexual stimulation or coitus, (2) known to effect fluid secretion across epithelia surfaces, or (3) known to alter fertilization success or coitus, (2) known to effect fluid secretion across epithelia surfaces, or (3) known to alter fertilization success. To test whether prolactin could mediate oviductal secretion, we asked what factors might mediate this increased oviductal secretion and flow. Genital stimulation and coitus evoke complex neurological responses. To simplify our task of determining factors that might increase oviductal flow, we considered known secreted hormones that were (1) elevated in serum after sexual stimulation or coitus, (2) known to effect fluid secretion across epithelia surfaces, or (3) known to alter fertilization success of sperm. Prolactin, in turn, counters dopamine’s mediation of sexual arousal and reduces sexual receptivity [23]. In humans, plasma prolactin concentrations are increased for >1 hr following orgasm induced by masturbation or sexual intercourse [24]. Female mice lacking the prolactin receptor gene have multiple reproductive defects, including reduced fertilization rate [25]. Finally, prolactin also has a plethora of effects on secretory epithelia [26]. To test whether prolactin could mediate oviductal secretion, we

Figure 1. Increased Fluid Production and Oviductal Flow after Mating
(A) The mouse uterus swells after mating. No fluid was recovered from the uterus at ovulation (i). Fluid from uteri removed immediately after mating increased by 37 mg, consistent with the weight (volume) of sperm fluid (ii). Fluid volumes increased markedly in uteri 8 or 12 hr after mating (120 or 100 mg, respectively) (iii and iv). Scale bar represents 1 cm.
(B) Accumulated uterine fluid was collected at the times indicated after mating. Fluid weights (volumes) from mated (red) and unmated (blue) females are plotted.
(C) Uterine fluid was collected from females 20 min (i), 4 hr (ii), or 8 hr (iii) after mating, and the fluids were centrifuged.
(D) Fluid flow in the oviduct (ampulla) 4 hr after mating. Migration of globular cells and debris was traced over 3 s; the closed circle marks the endpoint. The central diagonal structure is a mucosal fold in the oviduct wall. Oviductal isthmus is seen at bottom right. Scale bar represents 50 µm. See Movie S1.
(E) Average globular cell migration speed = 18.0 ± 1.6 µm/s at 22°C.
(F) Bromocriptine administration before mating inhibits fluid secretion measured 8 hr after mating.
(G) DsRed2-expressing sperm counts in the ampulla in vitro and ex vivo. Ex vivo: a portion of ampulla was dissected from females 4–5 hr after mating, and DsRed2-expressing sperm were counted. Oviducts were removed and ligated, or left unligated, near the UTJ 60–90 min after mating and then incubated at 37°C for 2–3 hr. The number of sperm reaching the ampulla in oviducts without an ovary averaged 31 ± 6. The increase in sperm number over the in vivo situation may result from manipulation or a change in hormone status perhaps detaching some sperm from the isthmal crypts and facilitating sperm migration. All graphs: mean ± SEM (black bars) and median with interquartile ranges (green boxes).
administered the dopaminergic receptor agonist bromocriptine (BrCr) 11 hr before coitus to inhibit prolactin secretion from the anterior pituitary. BrCr treatment reduced mouse uterine fluid accumulation (Figure 1F). In contrast, administration of the VR2 receptor antagonists oxytocin (atosiban) or vasopressin (tolvaptan) did not suppress oviductal secretion. We conclude that prolactin initiates the increased oviductal fluid flow to the uterus after coition. We next asked whether oviductal flow affected sperm movement.

Sperm Migration in the Oviduct
Ejaculated sperm migrate through viscous fluid to bind to epithelial cilia in the oviducal isthmus [6]. We tested whether sperm then detached from the cilia and swim to the ampulla after the mucus fluid had been cleared. Taking advantage of genetically modified sperm, in which enhanced green fluorescent protein (EGFP) is expressed in the sperm acrosome and DsRed2 fluorescent protein is expressed in sperm mitochondria [27] (Figure S1C), we found that 9 ± 1 sperm reached the ampulla 4–5 hr after mating (Figure 1G; in vivo). In order to test whether rheotaxis was functional ex vivo, oviducts were dissected from mice 60–90 min after mating. By this time, sperm had reached the isthmus, and in the next 2–3 hr, they migrated from the isthmus to the ampulla. Importantly, sperm in the isthmus migrated successfully to the ampulla ex vivo in the absence of an ovary, a source of potential chemottractants (Figure 1G). However, sperm transport was limited when oviducts were ligated near the uterotubal junction (UTJ) to block fluid flow (Figure S1B), as assessed by observation of DsRed2-tagged sperm upstream of the ligation site and by the lack of discharge of cell debris and globular cells. Sperm motility in the blocked oviduct appeared normal, but there were ~3-fold fewer sperm in the ampulla of the ligated oviducts (11 ± 2), indicating that fluid flow is a factor in optimal sperm transport ex vivo.

Spermatozoan Rheotaxis
The suggestion by several groups that transient receptor potential (TRP) channels are present in spermatozoa and might mediate sperm thermotaxis [28] motivated our initial studies to examine sperm movement in a thermal gradient. However, we found no effects on sperm motility or fertilization we noticed that temperature gradients create convection currents that influence the direction in which sperm swim. As the fluid in the oviduct is warmed from the bottom surface, fluid flows in well-recognized convection patterns, reaching a steady state in which fluid cycles from bottom to top and then down the sides of the chamber (Figure 2A). Sperm consistently swim against this flow (positive rheotaxis), whether along the top liquid surface (in the direction of increasing temperature) or bottom (in the direction of decreasing temperature) of the dish (Movies S2A and S2B). Solutions flowing through a pipette into a dish at constant temperature initiated identical positive rheotaxis (Movie S2C). To quantify rheotaxis, we defined rheotactic movement as movement within ±22.5° of the forward vector of sperm movement (swimming directly upstream; small particles defined the local vector of flow; Figure 2G). Providing the baseline in static media, we observe 12.5% of sperm in the field swim in the direction specified by the 45° arc centered along the sperm longitudinal axis. Without flow, mouse and human sperm swim in random directions (Figure 2H).

Rheotaxis of Uncapacitated and Capacitated Sperm in Low-Viscosity Media
Because sperm capacitation can vary with respect to position and time in the female reproductive tract [18], we evaluated sperm rheotaxis in both uncapacitated and capacitated mouse sperm. Ejaculated sperm swim to the oviductal isthmus through the UTJ within 1–2 hr, but most require several more hours to reach the ampulla [6]. When uncapacitated mouse or human sperm were added to the convective flow chamber, 68% and 51% of the actively motile sperm exhibited rheotaxis, respectively (Figures 2C–2F and 2H; Movies S3 and S4). To remove confounding results due to potential thermotaxis, we repeated these experiments in isothermal fields of flowing media. Uncapacitated sperm were placed in shear flow (Reynolds number [Re] = 0.002) via a 1 × 0.05 mm (w × h) capillary tube (Figure 2B). As expected, spermatozoa swim against the direction of flow (positive rheotaxis; Movie S2C), whereas in controls (no flow) sperm movement was randomly directed (Movie S2A).

Laminar flow in a chamber varies due to surface drag; velocity is highest in the center of the stream and falls to
a minimum next to the walls [34]. The flow gradient was quantified by measuring 1 \( \mu \)m spherical beads placed in the fluid as markers. Velocity profiles in fluid chambers are parabolic and approach linearly only near a small portion of their profile near a boundary. By tracking beads, we found that flow speed increased in the range of 0–300 \( \mu \)m from the surface. Bead velocity in the range of 0–80 \( \mu \)m could be approximated as \( \sim 1.45x \), where \( x \) is the distance in microns from the surface (least-squares \( r^2 = 0.98 \)). The swimming tracks of added sperm appeared as “threads” (similar to those in Figures 2D and 2F), swimming \( \sim 9 \) \( \mu \)m from the surface (flow velocity = 14.5 \( \mu \)m/s). In this low-viscosity fluid, capacitated mouse sperm that swim into the higher velocity of the central stream are swept downstream. Sperm swim near the surface where flow rate is slow, and those that stray away from the surface are swept away by the faster, more central flow under these conditions.

The lumen of the mouse oviductal isthmus consists of a narrow central channel surrounded by pockets formed by deep transverse mucosal folds. Sperm in the mucosal folds can access the central channel to migrate in the oviduct. To simulate the narrow central channel, sperm were capacitated for 2–3 hr in human tubal fluid (HTF) medium (cooled to 22 °C to limit sperm motility) and loaded into the capillary (Figure 3A; Movie S5A). The capillary was then transferred to a 37 °C chamber where sperm resumed more active motility. Under outward-directed flow (FlowOUT; \( \sim 15 \) nl/min or \( \sim 50 \) \( \mu \)m/s), sperm motility was recorded and sperm that had been swept out the end of the capillary counted. Whereas 44% of the sperm loaded into the tube were immobile, 78% of sperm swept from the tube were immobile (Figure 3B; Movie S5B). Conversely, when sperm were slowly sucked into the pipette (inward flow; FlowIN, \( \sim 15 \) nl/min or \( \sim 50 \) \( \mu \)m/s; 39% motile), 93% of sperm were active and moving against fluid flow, leaving immobile and some sluggish sperm behind (Figure 3B; Movie S6). Thus, gentle flow concentrates, or selects, viable sperm. Uncapacitated sperm showed essentially the same results (Figure 3B). In short, sperm swim upstream in a capillary mimicking flow rates in the oviductal lumen, a direction that also moves the oocyte toward the incoming sperm and eventually to the uterus. Thus, oviducal secretion and flow clear the oviduct and increase pH to induce hyperactivation; rheotaxis selects for motile sperm and ensures that eggs are fertilized while still in the oviduct. As proposed by Suarez and colleagues, slow release from reservoirs of quiescent sperm or sperm attached to epithelial or ciliary surfaces may provide a steady supply of sperm as this process is repeated over time [6].

**Sperm Movement in High-Viscosity Media**

During estrus, high-viscosity mucus is an obstacle to sperm progress through the isthmus lumen. Assuming, as a first approximation, that the fluid is Newtonian, we measured the viscosity of secreted and accumulated lumenal fluid in the uterus, in which oviductal mucus, cell debris, and ejaculated semen were present. The viscosities of this fluid averaged 81 ± 73 cP compared to the viscosity of clear fluid supernatants (2.4 ± 0.9 cP; Table S1). One caveat however, is that the fluid is thixotropic (viscosity decreases with shear) and thus likely non-Newtonian. Nevertheless, we estimate that the viscosity of lumenal fluid decreases to as low as 2–3 cP upstream of the congestion of mucus and cell debris in the isthmus. Therefore, we used media containing 0.3% methylcellulose (MC) (6.7 cP; Table S1) to mimic the environment of the oviductal lumen. As seen in Figure 4A and Movie S7A, uncapacitated mouse sperm swim in circles in viscous medium regardless of priming by bicarbonate ion in HTF medium (\( \sim 3 \) min), which increases flagellar beat frequency within 30 s [35]. The majority of uncapacitated sperm exhibit planar swimming (Figure 4A), with one side of the head facing the surface. This swimming behavior may increase the chance for uncapacitated sperm to stick to oviductal epithelial cells more frequently, a delay that might promote acquisition of the capacitated state [36].

**Sperm Rotate after Capacitation in Viscous Media**

When sperm are observed under phase-contrast microscopy, reflection intensity varies sinusoidally as the sperm head rotates, with the highest intensity corresponding to the head in the position vertical to the surface [13] (Figure 4C). We...
plotted sperm head reflection intensity as a function of time and call it “rotation frequency,” because it reflects the spinning of the sperm head and tail around a longitudinal axis. Rotation frequency was $1.7 \pm 0.1$ (SD)-fold higher in capacitated (Figures 4E and 4F) than uncapacitated (Figures 4D and 4F) sperm. During capacitation, the linear progress of the movement is increased by rotation (Figure 4B; Movie S7A). This motion may be boundary-driven (thigmotaxic) rather than rheotaxic. Sperm rotation after capacitation may enhance detachment from surfaces. Once in the stream, linear progress is enhanced; the head rotates and the sperm progression pattern oscillates around a motion-oriented parallel to the flow (Movie S8B). In summary, sperm rotation after capacitation enables sperm to swim into the main fluid stream (Figure 3C; Movie S8B).

response to fluid flow at a position perpendicular to the direction of fluid flow (11 out of 14 analyzed sperm; Figure 5B), followed by a return to normal rotation frequency (3.0 $\pm$ 0.2 Hz; SEM). Time-course analysis of turning sperm revealed a peak rotation rate of 7.1 Hz during turning, compared to 4.5 Hz prior to turning (Figure 5C, red). In contrast, the rotation frequency of sperm in steady-state positive rheotaxic movement was constant during steady flow and indistinguishable from sperm in “no-flow” conditions (Figures 5C and 5D). To further examine the propensity of sperm to turn into a stream we examined sperm at the confluence of two flow streams 45° apart (Figure 5E). In these situations, sperm rotation frequency significantly increased and resulted in sperm being directed into the stronger stream. Since inertia is negligible in the microscopic range [12] but governed by overdamped, zero Reynolds number dynamics, the system will be governed not by momentum, but rather by relative forces. Our conclusion is that sperm increase their rotation rate when they encounter tangential (side) forces.

Mechanisms: Tail Rotation Is Required for Rheotactic Movement
Orientation in a flowing stream has been described for mammalian sperm [11, 35] and bacteria [37]. Some mechanistic models of this orientation assume stronger hydrodynamic drag on the head (sperm) or cell body (bacteria) than the flagellum that orients the front of the cell toward the surface, resulting in the tail being dragged downstream with the head and cell body pointed upstream [11, 37]. To separate
the differential influence of drag on head and tail, we examined swimming headless sperm, which represent a minute fraction (<0.1%) of sperm from normal mice. Of these, 82% (14 out of 17) swim against fluid flow (Movie S10), indicating that the head does not act as an aft rudder and is not required for normal orientation—in other words, the rotating tail alone can exhibit positive rheotaxis.

Sperm lacking any one of five CatSper subunits (CatSpers1–4, CatSperΔ) exhibit identical phenotypes [38], fail to develop functional CatSper Ca2+ currents, and lack hyperactivated motility. Uncapacitated sperm lacking CatSper usually swim in a counterclockwise circular plane on the bottom surface, as observed from the top (Figure 6A; Movie S11A). This swimming behavior did not change regardless of fluid flow (Figure 6A; Movies S11B and S11C), indicating that the CatSper channel was required for effective rheotactic responses to fluid flow. Presumably, calcium influx through CatSper channels enhances sperm rotation, which changes circular planar movement to rotational swimming. Thus, rotation is an essential sperm motion for proper rheotaxis, and CatSper channels contribute to this response. Interestingly, S. droebachiensis sea urchin sperm did not swim against fluid flow; their trajectories were similar to those of mouse sperm lacking CatSper channels or uncapacitated sperm in high-viscosity medium (Figure 6B; Movie S12A). Regardless of fluid flow, these sea urchin sperm swim in circular planes (Movie S12B), consistent with the behavior of sperm from marine invertebrates that are not guided by marine currents but swim in concentric circles until encountering chemotactants that turn and widen their arcs of circular motion [39].

Prior studies show the sperm flagellum oscillates in a single plane when tethered at its head on a glass coverslip [35]. In order to better understand the motion of sperm in three dimensions during free swimming, we recorded a sperm flagellum every 50 ms and aligned these waveforms by horizontal translocation of the swimming direction axis (Figure 7A). During free swimming, flagella crossed the x axis at ~20% of their entire length as measured from the head (Figure 7A, right). This point is the minimum of flagellar excursion (Figure 7, arrowhead) and moves along the x axis with minimal y deviation or yaw into the plane (z axis). If sperm do not rotate, flagellar trajectories trace a triangular plane, resulting in minimal rheotaxis. Sperm rotation produces trajectories that trace a symmetric cone-like path (Figure 7B), consistent with previous observations of human spermatozoa that trace out a cone-shaped helical pattern and have an extended elliptical cross section when their heads are immobilized by attachment to a micropipette, but allow the tail to exhibit flexible three-dimensional movement [40]. Tangential forces on the tail anterior to the minimum of flagellar excursion will produce a clockwise force (as seen from above), whereas those on the posterior tail produce a counterclockwise force. The posterior tail receives stronger tangential force due to its higher proportion of the flagellar length (~80%), presenting a greater cross section to the force of fluid flow, pointing the sperm upstream (Figure 7B). If the sperm were fixed at the point of minimal excursion, this would be analogous to a weather vane that is
divided by an axis of rotation into a small anterior and a larger posterior surface that receives stronger tangential force and thus guides the weather vane into the wind. A freely motile sperm in a homogeneous flow is not constrained to be stationary at any point and therefore is not subject to torque, but it is subjected to differences in flow velocity along its length.

Discussion

We propose that rheotaxis, assisted by factors inducing hyperactivated motility (alkaline pH and, in humans, progesterone), is a major mechanism guiding sperm in mammalian female reproductive tracts. This rheotaxis is not related to sensory mechanisms and guidance responses as it is in large animals such as fish but a consequence of rotational motion potentiated by calcium entry via CatSper channels. Three arguments support sperm rheotaxis as a major guidance and selection mechanism. First, coitus triggers substantial fluid secretion into the oviduct, thus increasing lumenal flow directed by cilia and muscle contractions from ampulla to uterus. Second, positive rheotaxis of mature spermatozoa from mice and humans is observed in situ and in vitro. Finally, capacitating conditions and physiological fluid flow overcome the wall effect and sperm adhesion—these factors accelerate sperm rotation that in high viscosity fluid evokes a change from planar circular swimming to directional swimming. In contrast, the most popular hypothesis for taxis, chemotaxis, lacks both bona fide chemoattractant candidates and cognate receptors in mammals. We cannot, however, exclude a role for chemotaxis—if it occurs, it is most likely relevant over short distances. Similarly, we conclude that our results cannot be explained by thermotaxis. In the study of Bahat et al. [41], only ~5% of cells respond to temperature gradients, 8-fold less than the percentage of sperm responding to rheotaxis in our experimental system (~40% of the population; Figure 2).

Again, this does not mean that true thermotaxis or thermal effects on sperm motility are absent. Fluid secretion in response to coitus has been relatively unstudied. We found that there was little fluid secretion into the oviduct and uterus at ovulation in mice. In contrast, coitus induced a dramatic secretion of fluid that accumulated in the uterus (pH ~8.3), consistent with active extrusion of bicarbonate ion and water from the oviductal epithelium [42]. Interestingly, simulated coitus improves the success rate of artificial insemination in mice [43]. In humans, sexual stimulation of females increases serum prolactin [24], and our initial findings in mice suggest that this is mediated by bromocriptine-sensitive dopaminergic pathway control of the anterior pituitary gland. Prolactin regulates water and electrolyte balance in many organs (e.g., kidneys, fish gills [26]), perhaps via activation or regulation of the cystic fibrosis transmembrane conductance regulator (CFTR) [23, 42] or aquaporins [44]. With increased oviductal flow, globular cells from the oviduct were also discharged into the uterus within 4 hr after coitus. Apparently, ciliary motion and muscle contraction in the isthmus pushes oviductal fluid to the uterus, in turn washing away mucus and globular cells to clear the way for sperm traffic. Sperm harbored in the lower isthmus then orient and swim against this flow to reach the ampulla. Unlike the isthmus, countercurrents in the center of the ampullar lumen circulate fluid while ciliary currents push the fluid downstream into the oviduct [9]. Due to the hydrodynamic wall effect [10], sperm moving locally along ciliated epithelium may also swim against this ciliary current to reach the cumulus oophorus complex.
Sperm of marine invertebrates swim in a relatively unstructured three-dimensional space, where the encounter of an egg may be fortuitous. Chemotaxis substantially improves the odds of reproduction of these species. In contrast, the reproductive tract of mammals is restricted to a quasi-one-dimensional tube with increasing pH (and progesterone in humans) initiating hyperactivated motility to free sperm from adherent surfaces and rheotaxis to guide the sperm toward the oviduct. With up to 5 million mouse sperm deposited in the females after ejaculation, only ~20 sperm cells reach the ampulla within several hours, similar to the success rate of other mammals [40]. Oviductal flow has two advantages—it guides sperm to the egg but also selects for strong, capacitated sperm. Mammalian sperm must penetrate mucus, the viscoelastic matrix of the cumulus oophorus, and the tough zona pellucida, requiring vigorous sperm for successful fertilization. Also, directional flow sweeps the egg down the oviduct, and sperm that swim against the flow preferentially fertilize the egg while still in the oviduct. This provides time for the egg to develop for implantation. From this viewpoint, rheotaxis is a logical guidance and selection mechanism.

Our results suggest that positive rheotaxis is due to the spiral rotation of the sperm tail that orients sperm upstream, and as such, it is decidedly an active process. Calcium entry is required to trigger the increased amplitude of the distal tail excursion that increases tangential force. At the same time, increasing angular speed stabilizes the sperm’s swimming orientation due to increased motive force against the surroundings. Because the hyperactivated flagellar waveform is both asymmetric and of larger amplitude [18], the flagellum receives larger tangential forces, especially in high-viscosity solution [6], which points the sperm into flowing solution. Of course, the underlying basis of motive force and rotational motion is the basic adenosine triphosphate (ATP)/dynein motor; calcium modulates this motor and other components of the flagellar axoneme in ways that are not yet understood.

In summary, we find that coitus induces oviductal flow that clears the oviduct of cellular debris and provides rheotactic guidance for sperm. CatSper-mediated calcium entry associated with capacitation and subsequent hyperactivated motility potentiates and regulates the sperm rotation that is crucial to guidance in the graded, complex fluid flow in the oviduct.

Experimental Procedures

Mating and Collection of Uterine Fluid

All animal experiments were performed according to procedures approved by the Institutional Animal Care and Use Committee of Boston Children’s Hospital. Mice were housed in a 12 hr light (7 a.m.–7 p.m.)/dark cycle. Follicular growth and ovulation were stimulated in CD-1 female mice (Charles River) at 8 to 9 weeks of age by intraperitoneal (i.p.) injection of pregnant mare serum gonadotropin (5 IU, PMSG; Calbiochem) at 6:30 p.m., followed 48 hr later by i.p. injection of human chorionic gonadotropin (5 IU, hCG; Calbiochem). In some experiments, BrCr (10 mg/kg, B2134; Sigma) was subcutaneously injected 11 hr before mating. The mating period for B6D2F1 males was set between 6:20 and 7:00 a.m. the morning after hCG subcutaneously injected 11 hr before mating. The mating period for B6D2F1-Tg (B6D2F1-Tg; CAG/sufl-DsRed2, Acr3-EGFP RBG00200ab) enabled clear sperm visualization. Sixty to ninety minutes after the end of the mating period, oviducts were removed and ligated just after the UTJ (ovarian side). Oviducts were gently washed in HTF medium (Specialty Media, Millipore) to remove sperm attached to the outside wall and then incubated (5% CO2) for 2–3 hr. A portion of the ampulla was first cut with a fine scissors to prevent sperm from leaving the isthmus, then the oviduct was stretched by tracing the mesosalpinx and placed on a glass slide (14 each of ligated or unligated oviducts), and sperm inside were counted under fluorescence microscopy. Samples in which there were obvious sperm aggregates on either side of the ampulla were removed from statistical analysis (two cases each; ligated and unligated oviducts).

Sperm Preparation

Mouse sperm were collected from the B6D2F1 male (Jackson Laboratories) caudal epididymis and incubated for 5 min to allow sperm to swim out. Cryopreservation at 900 g (5 s) removed debris and laminated sperm aggregates. Sperm were then incubated at a density of 2 × 106/ml in M2 media for 30 min or HTF medium for 2–3 hr (5% CO2), yielding uncapacitated or capacitated sperm, respectively. Cryopreserved human sperm were purchased from Fairfax Cryobank. Actively motile human sperm were collected by the swim-down procedure. Frozen sperm (~3 × 105) in egg yolk were thawed and washed by incubation in 5 ml HTF, followed by centrifugation at 500 g × 5 min to obtain a sperm pellet. After two rinses, the pellets were resuspended in 0.5 ml HTF. Half of the sperm suspension was overlaid with a 0.5 ml Percoll cushion (80% HTF, 20% Percoll, 0.1 M NaCl). Sperm were incubated for 45 min in a 37°C/C02 incubator and sperm swimming-down into a Percoll cushion were recovered by centrifugation at 1,800 g × 1 min. Sperm in the pellet were resuspended in 0.5 ml HTF and incubated for 30–60 min.

Green sea urchin (Strongylocentrotus droebachiensis; IFST Trading, Marine Biology Laboratory, Woods Hole, MA, USA) sperm were obtained by intracoelomic injection of 0.5 M KCl. Eluted sperm were suspended in artificial seawater (ASW) containing 486 mM NaCl, 30 mM MgSO4, 10 mM MgCl2, 10 mM KCl, 10 mM CaCl2, 2.5 mM NaHCO3, and 10 mM HEPES (pH 8.0).

In Vitro Sperm Rheotaxis

Sperm rheotactic movement was analyzed in convective and capillary flow. For convective flow, 2 ml of culture medium and 1–2 × 105 mouse sperm or 2–4 × 105 human sperm were added to the 37°C chamber (Delta TC culture dish controller; Bioptechs). M2 or HEPES-HTF medium (92 mM NaCl, 2 mM CaCl2, 4.7 mM KCl, 0.2 mM MgCl2, 0.37 mM KH2PO4, 25 mM NaHCO3, 18.3 mM Na lactate, 2.78 mM glucose, 0.33 mM Na pyruvate, 0.4% [w/v] bovine serum albumin [BSA], and 10 mM HEPES [pH 7.4]) was used for mouse or human sperm, respectively. In some experiments, the medium was supplemented with 0.3% [w/v] methyleneblue (MC) (M0512, 4,000 C/P in 2% solution; Sigma). Sperm swimming 3–5 mm from the rim were recorded by CCD camera (4 or 10 × objective, mouse or human sperm, respectively; KP-F31SCL; Hitachi) after a 10 min preincubation period that allowed spontaneous dissociation of sperm clumps and stabilized convective flow. For control (no flow) and capillary flow experiments, 1 ml of medium was overlaid by 1 ml of mineral oil and covered by a heated glass lid (Bioptechs) to inhibit convection. A glass pipette of 1.2 mm (OD) rectangular glass capillary (~50 μm [h] × ~1 mm [w]) was placed on the bottom of the chamber, and the media was gravity fed at 1.5 ml/hr. Mouse sperm (1–2 × 106) were transferred to the heating chamber and analyzed for sperm rheotaxis at a constant 37°C. For sea urchin sperm, 2 ml of ASW and 1–2 × 106 sperm were added to a 35 mm culture dish precoated with 0.1% gelatin to prevent sperm surface adhesion. ASW containing 1 μm microbeads (Micromer; Micromod) was perfused at 3 ml/hr (22°C).

Measurement of Sperm Rheotaxis

Sperm movement in 3 s intervals was recorded by CCD at 60 frames/s, processed using digital image processing software (XCAP; EPIX), and analyzed using ImageJ. Swimming trajectories were via Computer Assisted Sperm Analysis (CASA; http://rsweb.nih.gov/ij/plugins/casa.html). Sperm
movement within ±22.5° from the vector –180° from the direction of fluid flow was defined as rheotactic movement (Figure 2G). Sperm undergoing rheotaxis were divided by the number of motile sperm; sluggish sperm, swept out by convective flow, were excluded from the count.

Uncapacitated and Capacitated Sperm Movement in the Capillary Tube Mouse sperm incubated in the HTF medium for 2–3 hr or M2 medium for 30 min at 2 × 10^7/ml yielded capacitated or uncapacitated sperm, respectively. Sperm were centrifuged at 900 g x 1 min, resuspended in HEPES-HTF or M2 medium with or without 0.3% (w/v) MC at 4 x 10^7/ml, and loaded into the capillary by suction via an air-pressure microinjector (IM-58; Narishige; 22°C). Sperm in the capillary were transferred to the 37°C chamber containing 2 ml of HEPES-HTF or M2 medium with or without 0.3% MC at 37°C (~150 sperm for each experiment). In some experiments, microbeads (1 μm) served as a flow indicator; a flow rate of ~3 μl/mm/min provided ~50 μm/sec flow in the cross-section imaged.

Viscosity Measurements

The viscosity of luminal fluid and media was measured using the rolling-ball method at 22°C. Variable glycerol concentrations were used as calibration standards.

Statistical Analysis

Data analyses were performed using Microsoft Excel and GraphPad Prism. Statistical Analysis standards.

Supplemental Information

Supplemental Information includes one figure, one table, and 12 movies and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.02.007.

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