Collective sperm movement in mammalian reproductive tracts

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Abstract

Mammalian sperm cells travel from their origin in the male reproductive tract to fertilization in the female tract through a complex process driven by coordinated mechanical and biochemical mechanisms. Recent experimental and theoretical advances have illuminated the collective behaviors of sperm both *in vivo* and *in vitro*. However, our understanding of the underlying mechano-chemical processes remains incomplete. This review integrates current insights into sperm group movement, examining both immotile and motile states, which are essential for passive transport and active swimming through the reproductive tracts. We provide an overview of the current understanding of collective sperm movement, focusing on the experimental and theoretical mechanisms behind these behaviors. We also explore how sperm motility is regulated through the coordination of mechanical and chemical processes. Emerging evidence highlights the mechanosensitive properties of a sperm flagellum, suggesting that mechanical stimuli regulate flagellar beating at both individual and collective levels. This self-regulatory, mechano-chemical system reflects a broader principle observed in multicellular systems, offering a system-level insight into the regulation of motility and collective dynamics in biological systems.

1. Introduction – Sperm journey to meet with egg in reproductive tracts

In mammals, the journey of sperm from their production to fertilization is an intricate process marked by a series of coordinated events within both male and female reproductive tracts (Figures 1A–B). Physiological and molecular mechanisms underlying these events have been extensively studied using model animals, such as mice and rats. Spermatogenesis occurs in the seminiferous tubules of the testes, where spermatogonia progressively transform into differentiated sperm cells through the sequential stages of mitosis, meiosis, and spermiogenesis [1]. After release from the Sertoli cells – a process known as spermiation – sperm are transported via luminal fluids to the rete testis, efferent ducts, and eventually to the epididymis [2]. In the epididymis, sperm undergo further maturation, gaining motility and the capability to fertilize an egg or ovum. This maturation involves characteristic protein modifications and plasma membrane remodeling [3,4]. Once matured, sperm are stored in the cauda epididymis until ejaculation. During ejaculation, sperm are propelled through the vas deferens and urethra into the female reproductive tract, accompanied by seminal fluid that provides supportive nutrients and protection against the acidic environment of the vagina [5]. The vigorous beating of the flagellum is crucial for sperm to navigate through the cervix and uterus, eventually passing through the utero-tubal junction (UTJ) to enter the oviduct, also known as the fallopian tube or the uterine tube, where fertilization usually occurs. Upon entering the oviduct, sperm undergo capacitation—a process characterized by biochemical changes, including membrane destabilization and an increase in intracellular calcium [6]. After migrating through the UTJ, the sperm attach to the oviductal reservoir and are then released from it due to physiological changes during capacitation [7]. These processes are crucial for the acrosome reaction, during which enzymes are released to penetrate the zona pellucida of the egg, although enzyme release may be dispensable for sperm penetration into the zona pellucida in mice [8,9]. The later stages of fertilization encompass the recognition and binding of sperm to the surface proteins and receptors of eggs, culminating in the fusion of sperm and vitelline membrane. This event initiates egg activation and the onset of zygotic development, marking the conclusion of the sperm journey from origin to fertilization.

Throughout their journey, the sperm movement can be broadly categorized into three phases: passive, active, and hyperactive [10] (Figure 1C). The transition from passive to active movement is considered to occur before reaching the distal corpus of the epididymis in mice and rats [11,12], indicating that sperm are passively transported from the seminiferous tubules to caput epididymis. The mechanisms of passive transport, involving hydraulic forces within the luminal environments, have been previously discussed earlier [2,13]. Once capable of active movement, sperm undergo a crucial transformation in the oviduct known as hyperactivation. This process significantly alters the motility pattern of sperm, characterized by larger amplitude of flagellar beating and vigorous lateral head displacements [14]. Hyperactivation enhances fertilization efficiency by enabling sperm to navigate the complex environment of the female reproductive tract and penetrate the surrounding barriers of eggs.

This review focuses on collective sperm behaviors within mammalian male and female reproductive tracts, with a particular emphasis on studies involving mice and rats. The aim is not only to complement and enhance the understanding of sperm cell dynamics, summarized in an earlier review [15], but also to provide new perspectives on emergent properties of sperm collectives in *in vivo* contexts. We begin by examining current understandings of collective sperm movement observed both *in vivo* and *in vitro*, offering insights into the mechanisms behind these behaviors from experimental and theoretical perspectives. We then explore how sperm motility is regulated through the coordination of mechanical

and chemical processes. Finally, we discuss a potential self-regulatory system underlying collective sperm movement, proposing it as a general principle in multicellular systems.

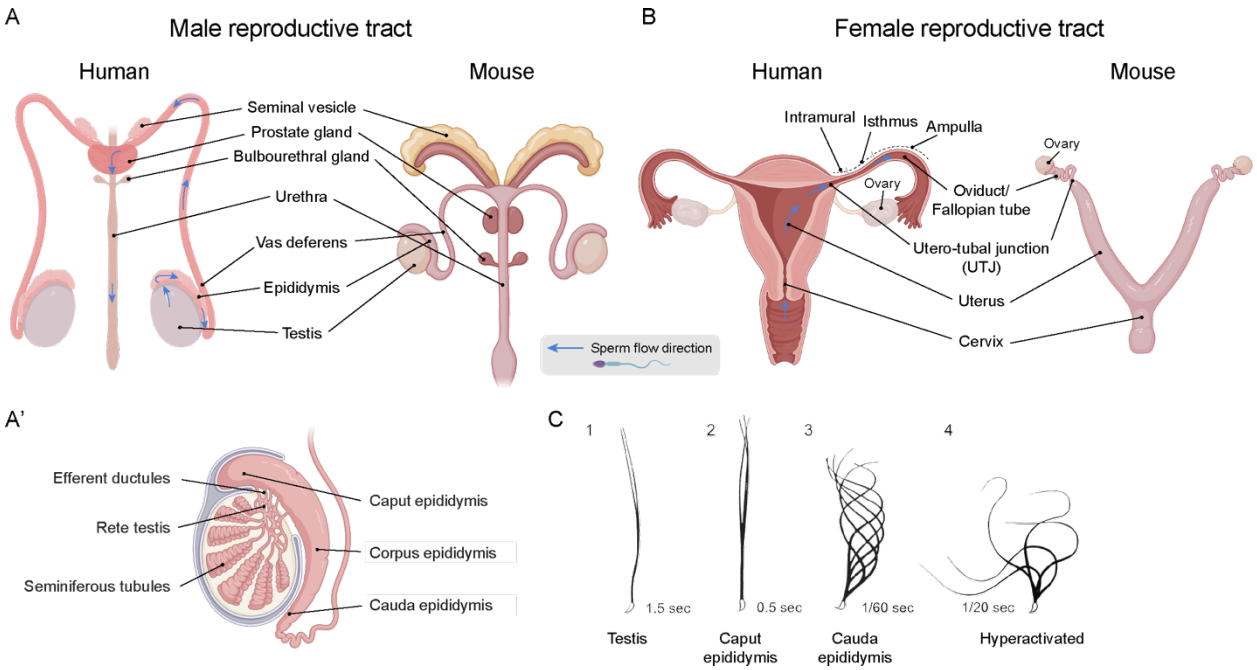

Figure 1 Sperm flow motion in reproductive tracts

(A–B) Sperm flow in the male reproductive tract (A) and the female reproductive tract (B) of humans (left) and mice (right). Blue arrows indicate the direction of sperm flow within the tracts. (A') Schematics of proximal organs in the male reproductive tract. Illustrations were created with BioRender.com, and certain aspects do not accurately reflect the actual size and shape.

(C) Flagellar movement of golden hamster sperm from the testis (1), caput epididymis (2), cauda epididymis (3), and during hyperactivation (4). The time intervals between successive tracings are indicated. Reproduced from [10] with permission.

2. Collective sperm behaviors *in vivo* and *in vitro*

This section discusses collective sperm behaviors observed *in vivo*, some of which have only recently been elucidated through advances in imaging technologies. To advance the understanding of these behaviors, particularly sperm clustering, we present *in vitro* observations and provide theoretical interpretations of the underlying phenomena.

2.1. Direct observation of *in vivo* sperm behaviors

The transport of immotile sperm from the seminiferous tubules to the epididymis within the male reproductive tract is primarily driven by the extracellular flow of luminal fluids. This luminal flow is regulated by multiple factors, including the coordinated secretion and reabsorption of intraluminal fluids, pulsatile ductal contractions, and whip-like beating of cilia on the epithelial cells of efferent ducts [2,13]. Intravital imaging reveals that flagella of spermatids align along with testicular fluid flow within the lumen in mice (Figure 2A)[16]. Additionally, the orientation of sperm flagella, which project from the seminiferous tubules into the lumen, undergoes complete reversals in response to testicular luminal flow within a minute. This dynamic flow not only facilitates the downstream transport of sperm to the rete testis but also potentially supports efficient spermiation through fluid shear stress.

A recent study introduced an *in vivo* imaging technique using optical coherence tomography (OCT) for live three-dimensional visualization of the mouse testis and epididymis [17]. This approach effectively captures the dynamic contractility of tubules and sperm transport within these structures, revealing significant variations in contraction frequencies across different regions of the epididymis. The highest contraction frequency was observed in the caput region of the epididymis, gradually decreasing toward the cauda. The caput epididymal duct exhibited the highest contraction frequency (51.9×10^{-3} Hz, averaging 19 seconds per contraction), likely facilitating the rapid initial transport of sperm. As sperm progress toward the cauda, the contraction frequency diminishes (10.9×10^{-3} Hz, averaging 92 seconds per contraction), potentially reflecting the need for slower transport and prolonged storage of sperm. While this OCT imaging technique provides valuable quantitative data under physiological conditions without the need for specific cellular labeling, its maximum spatial resolution of 4 μm is insufficient to detect single sperm behavior in dense conditions. This limitation highlights the need for further advancements in live cell imaging techniques to explore collective sperm behaviors in the reproductive tracts in greater detail.

Several imaging studies have demonstrated that motile sperm form clusters within the mouse female reproductive tract. The *in vivo* OCT imaging technique mentioned above has revealed that sperm clustering and subsequent separation occur in the oviduct ampulla, where fertilization takes place [18]. Fluorescence live imaging under *ex vivo* conditions has shown that mouse sperm migrating from the uterus tend to aggregate in the isthmus, with this clustering being highly correlated with the shuttling flows of oviductal fluids [19]. Furthermore, the formation of sperm clusters at the UTJ shortly after mating has been confirmed through tissue-clearing and 3D imaging techniques [20] (Figure 2B, B'). In the uterus and UTJ, sperm attach to the epithelium via the sperm hook, potentially facilitating unidirectional movement [21]. Interestingly, the transient joint movement observed during sperm clustering exhibits sharp increase in instantaneous sperm swimming speed, which drops back as the cluster separates [18]. This clustering behavior may enhance sperm motility through collective dynamics, facilitating navigation towards and entry into the oviduct, potentially increasing the success rate of fertilization. However, it has been reported that the beating speed of sperm flagellum slows down in the intramural UTJ, possibly due to the mechanical resistance of intraluminal fluid [22]. These findings highlight the importance of not only collective features of motile sperm but also the mechanical environment in influencing sperm motility.

2-2. Motile sperm clustering and aligning

One of the key factors promoting sperm clustering is the rheological properties of the surrounding fluids. Tung et al. revealed that dynamic clusters of bovine sperm *in vitro* form with sperm aligning and orienting in the same direction within each cluster in a viscoelastic medium [23]. They found that the frequency and size of sperm clusters, as well as the strength of sperm alignment, increase in viscoelastic fluids but not in purely viscous fluids. This suggests that the elastic component of the fluid plays a crucial role in facilitating sperm-fluid interaction and enabling collective swimming patterns. Additionally, they observed that sperm dynamically move in and out of clusters, as has been reported *in vivo* in mice [24], indicating that clusters are not static but rather form and disband within this fluidic environment that mimic the viscosity of the female reproductive tract [25]. When transitioning from a low-viscosity (15 cP) to a high-viscosity medium (100 cP), sperm tend to attach at the head region and swim as a cooperative group. Within this range, sperm form motile swimming clusters but slow down as the viscosity increases. However, when in clusters, their swimming efficiency increases by over

50% compared to individual sperm, which is consistent with the behavior observed in bovine sperm [23]. Under physiological conditions, sperm at the entrance to the uterus should navigate through viscoelastic mucus and other mucoid secretions to reach the site of fertilization, necessitating a mechanism adapted to these complex fluids for efficient movement and successful fertilization.

What drives the clustering of motile sperm? Theoretical studies suggest that the flow fields generated by sperm flagellar beating drive sperm clustering by aligning them side by side in low Reynolds number fluids, where viscous forces dominate over inertial forces. In viscous fluids, 'pusher' type swimmers, such as typical mammalian sperm or bacteria with rear-mounted flagella, push fluid away from their tails, creating a flow field that propels them forward while drawing fluid inward from the sides. These longitudinal outward and lateral inward flows, locally created by the beating of a single sperm flagellum, help in aligning and attracting other sperm, leading to the formation of swimming clusters [26–28]. Conversely, 'puller' type swimmers, such as algae with front-mounted flagella, pull fluid towards their front, generating a flow field that moves them forward while expelling fluid from the sides, which repels sperm if they are in close proximity. Notably, sperm can transiently switch from pusher to puller types of motion depending on mechanical factors, such as fluid rheology, the presence of boundaries, and interactions with other cells.

Agent-based simulations have shown that multiple motile sperm tend to align and bundle together over short distances as they swim in two-dimensional space through hydrodynamic interactions [29,30]. Furthermore, Ishimoto et al. demonstrated that a viscoelastic fluid, similar to that used experimentally [23], can enhance sperm clustering compared to a low-viscosity medium [31,32]. One interpretation for this enhanced clustering is that high viscous resistance suppresses both sperm flagellum yaw, which would otherwise push them apart, and the spontaneous switching between pusher and puller swimming modes. This suppression allows sperm to remain in close proximity within clusters. Taketoshi et al. provided a detailed examination of the fluid-structure interactions between two sperm swimming in parallel, revealing that their swimming speed increases due to enhanced fluid flow without any alteration in the beating patterns of their flagella [33]. Overall, these findings underscore the hydrodynamic effects of cooperative sperm swimming.

Sperm clustering is influenced not only through hydrodynamic interactions driven by flagellar beating but also by the levels of DNA fragmentation and the composition of the plasma membrane. Xiao et al. reported that human sperm swimming collectively exhibited lower levels of DNA fragmentation and higher cholesterol content in the plasma membrane compared to solitary swimming sperm [25]. They also found that sperm exhibiting planar swimming, which results in faster movement, had lower DNA fragmentation compared to those exhibiting bulk swimming [34]. Although correlations exist among sperm swimming modes, DNA integrity, and plasma membrane composition, the causal relationships remain unclear. It is possible that specific adhesion molecules on the plasma membrane, associated with changes in cholesterol composition, play a role in the transient maintenance of sperm clustering.

2-3. Dynamic sperm movement in dense suspension

When motile active matter is in a dense suspension, it creates coherent motion in a self-organized manner—a fundamental characteristic observed across various scales, from molecular assemblies to larger biological entities. Particularly, when filamentous or elongated motile structures are densely suspended, they generate complex flows characterized by swirling and turbulence, highlighting the intricate dynamics within active matter systems [35,36]. This collective behavior highlights how

individual movements contribute to emergent macroscopic phenomena, as demonstrated by various systems, including cytoskeltons-motor proteins [37,38], bacteria [39,40], mammalian cells [41,42], and roundworms [43].

Earlier experiments regarding *in vitro* collective sperm movement have demonstrated that sperm exhibit rich spatiotemporal patterns when in dense suspension. Riedel et al. found that sperm form dynamic vortices exhibiting quantized rotating waves, akin to turbulent fluid flows, at a critical sperm density [44]. The interactions between the fluid motions generated by the flagella of closely positioned sperm lead to a self-organized pattern where sperm align their swimming paths and synchronize their movements, enhancing their swimming efficiency through large-scale coordination. Similarly, Creppy et al. observed swirling and complex flow behaviors using ram semen samples under concentrated sperm conditions in a chamber sandwiched between two glass plates [45] (Figures 2C, C'). Through statistical analysis, they confirmed that sperm flow patterns exhibit quasi-2D turbulence. These studies provide strong evidence that motile sperm behave as swarming liquid crystals, characterized by rich and coherent patterns such as swirls, vortices, and waves, resulting from the interactions of constituent agents.

The collective behaviors of sperm in densely populated regions within the reproductive tract, such as the cauda epididymis or proximal vas deferens, remain poorly understood due to limited imaging studies. Previous research has shown that sperm extracted from the murine epididymis display varied motility characteristics depending on their region of origin. Murine sperm derived from the distal caput exhibit irregular motions with limited forward movement, whereas their motility is significantly enhanced in the corpus region [11]. These findings imply that sperm acquire their self-propelling capacity between the caput and corpus regions, with full motility being established by the time they reach the cauda epididymis. Despite their motility, *in vitro* studies have surmised that sperm stored in the cauda epididymis remain quiescent [46,47]. However, fixed tissue sections from the murine cauda epididymis suggest characteristic patterns of sperm alignment under dense conditions (Figure 2D). Given the motility of sperm in these regions, their self-propelled nature as active matter and the emergent properties resulting from many-body interactions likely play critical roles in their collective dynamics.

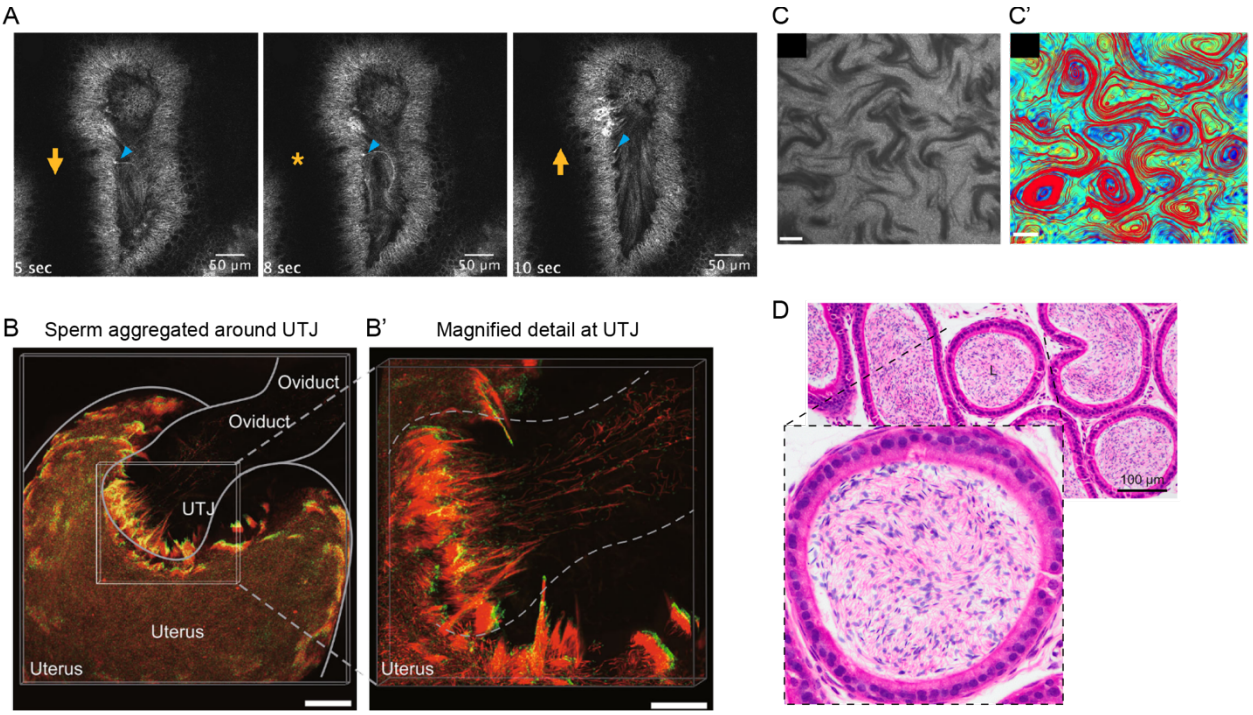

Figure 2: Collective sperm behaviors *in vivo* and *in vitro*

(A) Time-lapse *in vivo* fluorescence images of sperm flagella on the luminal surface of a seminiferous tubule. The direction of the flagella (orange) on the seminiferous epithelium switches within a minute. Blue arrowheads indicate the sperm head. Scale bars, 50 μm. Reproduced from [16].

(B, B') 3D imaging of sperm aggregation around the murine UTJ, with GFP-labeled acrosome (green) and RFP-labeled mitochondria (red). Scale bars, 200 μm. Reproduced from [20] with permission.

(C, C') Phase-contrast image of ram semen (D) and the normalized color-coded vorticity field with instantaneous velocity streamlines (red) (D'), placed between two glass plates *in vitro*. Scale bars, 200 μm. Reproduced from [45] with permission. The original indices in the top left corner of each panel are blacked out.

(D) Paraffin section stained with hematoxylin and eosin of the murine cauda epididymis. Scale bar, 100 μm. Reproduced from [13] with permission.

3. Mechano-chemical system for sperm flagellar beating

Mammalian sperm propel themselves forward using a flagellum, which extends from the cell body—measuring approximately 120 μm in mice and 50 μm in humans [48]. This active motion is powered by the conversion of chemical energy obtained from the environment into mechanical motion at the molecular level, driving the rhythmic beating of the flagellum. In this section, we explore these processes in reverse order—beginning with the mechanical molecular action for flagellar beatings and tracing back to the energy metabolism, enabling us to identify key factors by connecting outcomes to their causal inputs.

3-1. Molecular machineries in axonemes

The propulsive oscillations of sperm flagella are orchestrated by the synergistic interplay among microtubules, the motor protein dyneins, and other structural proteins [10,49]. Central to this dynamics is based on the axoneme, the core structural component of sperm flagellum, which typically consists of nine peripheral doublet microtubules circumferentially arrayed around a pair of central single microtubules, referred to as the "9+2" pattern. Within this axonemal architecture, the dyneins, which

connect between neighboring doublet microtubules in concert with regulatory complexes of nexin linkages, orchestrate the bending motions essential for the sperm flagellar beatings through the conversion of microtubule-dynein sliding [50,51]. In addition, radial spokes, which extend from the doublet microtubules to the central pair of microtubules, play a pivotal role in modulating the flagellar beating patterns. Even in the absence of central microtubules, sperm can vigorously beat their flagella with helical movements as observed in the 9+0 axonemal structures of certain organisms such as eels [52] and the Asian horseshoe crab [53]. This indicates that the central pair of microtubules is not necessary for flagellar beating but may control it. Accumulated observations suggest that the sliding activities of dyneins are not uniformly enhanced; instead, they appear to be regulated in a spatiotemporally coordinated manner to generate the localized curvatures that characterize flagellar motion [54,55]. To enhance our understanding of the non-planar and asymmetric patterns of sperm flagellar beatings observed in mammals, such as mice and humans [56–58], detailed analyses are needed to elucidate further molecular components involved within the axoneme and how these axonemal molecular machineries respond to various stimuli in a coordinated manner to produce propulsive flagellar oscillations.

The innovative application of cryo-electron tomography (cryo-ET) has recently provided comprehensive structural insights into the molecules within sperm axonemes [59–62]. Chen et al. utilized in situ cryo-ET to identify asymmetric distributions of non-motor protein complexes, such as the radial spokes and the nexin-dynein regulatory complexes, across the cross-section of mammalian sperm axonemes [59]. They propose that these asymmetric distributions collectively enhance the mechanical strength at the supra-molecular scale, enabling resistance to flagellar bending and underpinning the characteristic movements of the sperm flagellum. Furthermore, the study revealed variations in the asymmetric distribution of these components between mice and humans, suggesting structural foundations for distinct swimming patterns across species. In a complementary study, the same team demonstrated that cryo-ET analysis, when combined with AlphaFold2 docking analysis, could reveal the presence of unique, sperm-specific protein complexes associated with microtubules in the sperm flagellum [60]. They successfully identified novel microtubule-associated proteins in mammalian sperm, such as Tektin 5, CCDC105, and SPACA9, which enhance the structural integrity and mechanical properties of the sperm flagellum through extensive interaction networks within the axonemal microtubules. This work exemplifies the potential for further systematic characterizations of molecular architectures, particularly when integrated with CRISPR/Cas9-mediated knockout approaches in mice [63–65], paving the way for complete elucidation of sperm functionality at the molecular level.

Among the crucial axonemal factors, dyneins tethered to doublet microtubules play a pivotal role in the beating of the sperm flagellum. These dyneins require ATP hydrolysis to provide the energy required for their arms, generating minus end–directed force along microtubules [66,67]. They consist of various types of heavy chains, each encoded by different genes, exhibiting unique motor and ATPase characteristics. The motor units within the same complex are regulated by distinct signaling inputs. However, ATP alone is insufficient to induce sperm flagellar beating. Classic studies showed that sperm initially remained immotile when placed in a medium containing only ATP, although they eventually began to exhibit motility; introducing cAMP to the ATP-containing medium triggered immediate motility in various animals including some mammals [68,69], indicating that cAMP serves as the primary initiator of axonemal movement in mammalian sperm. cAMP signaling pathway regulates sperm motility through phosphorylation of dynein light chains and other axonemal proteins

[49,70], even though the cAMP signaling pathway may be unnecessary for fertilization in mice [71]. The phosphorylation can alter the conformation and interaction of dyneins with axonemal microtubules, thereby affecting the sliding mechanism that drives flagellar beating. In addition, tubulin polyglutamylation and glycylation have been shown to control flagellar beating and patterns, involving male fertility [56,72]. These regulations allow sperm to respond to physiological signals, coordinate microtubule-dynein interplay, and adapt to changing environmental conditions mediated through the cell signaling system [73,74].

3-2. Signal transduction to mechanical actions

The level of cAMP within a sperm axoneme is regulated by adenylyl cyclases (ACs), which catalyze the conversion of ATP to cAMP, releasing pyrophosphate in the process. Mammalian ACs include two distinct types: transmembrane ACs and soluble ACs (sACs) [75]. Genetic studies have revealed that sAC in murine sperm mediates several signaling events crucial for sperm motility and fertility [76,77]. This discovery has opened avenues for effective pharmacological targeting in male contraception [78]. Soluble ACs are directly activated by essential ionic regulators, such as Ca^{2+} and bicarbonate, thereby serving as key mediators of these signaling inputs in various regions within the axoneme. In sperm, sACs are the main source of cAMP, activating the downstream cAMP-dependent protein kinase or protein kinase A (PKA) pathway, which modulates flagellar beating [79,80].

As described in the section 3-1, the sliding activities of dyneins should be spatio-temporally controlled to generate propagations of the localized curvatures on sperm flagellum. Given that PKA activation regulates the sperm flagellar movement at molecular scale [81,82], the PKA-mediated pathway should possess regulatory mechanisms that ensure precise input-output dynamics. Considering purely physical diffusion properties, the catalytic domain of PKA has a radius of approximately 50 Å, making it about 50 times larger than small signaling mediators such as Ca^{2+} [83]. According to the Stokes-Einstein relation, this size disparity suggests that the diffusion coefficient of PKA is over 50 times slower than that of smaller molecules in a simple regime. Consequently, for rapid and spatially precise signaling, the activated form of PKA is likely localized in close proximity to axonemal dyneins. Supporting this notion, immunoelectron microscopy has revealed that the catalytic subunit of PKA localizes to the outer arm dynein of flagellar axonemes in rainbow trout sperm [84]. This also support, though in an indirect way, that the active form of PKA regulates dynein-mediated sliding.

Despite significant efforts to characterize the signaling pathways in *in vitro* single sperm [49,85], the detailed mechanisms of signal modulation within sperm as they traverse the reproductive tracts remain largely unresolved. The sAC–cAMP–PKA axis is one of the main pathways for the regulation and maintenance of the distinct modes of sperm flagellar movement (Figure 1C). The transition from immotile to motile is particularly intriguing, occurring as sperm pass through the epididymal duct and acquire motility within the epididymis. Given the involvement of this motility-related signaling system, it is reasonable to hypothesize that PKA activity in sperm increases as they progress from the caput to the corpus, although direct experimental evidence is currently lacking.

3-3. Energy metabolism for flagellar beating

Mammalian sperm generate ATP through two primary metabolic pathways—oxidative phosphorylation (OXPHOS) and glycolysis—to sustain the energy-intensive process of motility. OXPHOS is localized to the midpiece, where mitochondria produce ATP with high efficiency by harnessing the energy derived from electrons transferred along the electron transport chain. In contrast, glycolysis

predominates in the principal piece of the flagellum, where ATP is synthesized on-site through the breakdown of glucose, directly supporting the high energy demands of flagellar movement. This dual metabolic strategy enables sperm to dynamically respond to varying energetic requirements, utilizing both endogenous reserves and exogenous substrates available within the male and female reproductive tracts [86,87].

The two metabolic pathways possess distinct characteristics, which would allow them to complement or synergize with one another to optimize ATP production under varying conditions. While mitochondria are considered the powerhouses of sperm to generate ATP through OXPHOS, the efficiency of ATP diffusion from the midpiece to the distal tip of the flagellum remains an open question. Given the length of the mammalian flagellum, e.g., approximately 120 μm in mice, simple diffusion alone may be inadequate to meet the rapid kinetics required for the propagation of flagellar waves [86,88]. On the other hand, glycolysis, which generates ATP directly within the flagellum, likely plays a pivotal role in sustaining the high energy demands of the microtubule-dynein sliding along the axoneme. This process predominantly takes place in the principal piece of the flagellum, where glycolytic enzymes should be positioned to produce ATP where it is most needed. This localized production ensures immediate ATP availability, circumventing the potential limitations imposed by diffusion from the midpiece. Studies have demonstrated that inhibiting glycolysis markedly impairs sperm motility, even in the presence of mitochondrial substrates, highlighting the indispensable role of glycolysis in sperm energy metabolism [89,90]. Accumulated evidence indicates that while both OXPHOS and glycolysis support mouse sperm motility, the glycolytic pathway is required for the hyperactivation [87,91]. Given that glycolysis occurs in the cytoplasm, an intriguing open question is how efficient this process would be within the relatively sparse cytoplasmic environment of the sperm principal piece.

It remains incomplete how sperm utilize endogenous energy stores and acquire exogenous substrates within the reproductive tracts. While some studies suggest the presence of glycogen stores in mammalian sperm and propose the potential for gluconeogenesis, where glucose is synthesized from non-carbohydrate sources like amino acids, lactate, and glycerol [88], the evidence is not yet conclusive. It is well established that sperm uptake energy sources and receive macromolecules that activate signaling pathways crucial for hyperactivation and sustained motility as they traverse the female reproductive tract [85,92]. However, the understanding of how chemical signals are transmitted from somatic cells to sperm within the male reproductive tract is limited. Recent studies have demonstrated that the mammalian epididymis is highly responsive to acute dietary changes [93], acting as a mediator organ that transmits diet-induced metabolic alterations to sperm as they transit through the epididymal duct [94]. This process can lead to mitochondrial dysfunction in sperm during maturation [95]. The transfer of this metabolic information is facilitated by epididymosomes – extracellular vesicles secreted by the epididymal epithelium that carry a cargo of proteins, RNAs, and metabolites [96,97]. The next challenge lies in elucidating the precise mechanisms by which these extracellular vesicles influence sperm metabolism and functionality.

4. Multicellular feedback regulation for collective sperm movement

As discussed in the previous section, the molecular mechanisms that fuel microtubule-dynein sliding for sperm flagellar beatings have been extensively studied. However, our understanding of the input-output system that control the spatio-temporal patterns of oscillatory flagellar beatings remains

incomplete. This section focuses on the mechanosensing and response mechanisms of the sperm flagellum, aiming to propose a collective feedback system.

4.1. Experimental insight into self-regulatory flagellar beating

Recent studies have begun to provide experimental evidence on how the coordination of the microtubule-dynein sliding is achieved during sperm flagellar beating. For example, a cryo-ET study demonstrated that outer arm dyneins are inactive on one side of the bent flagellum across the cross section while those on the opposite side are active in sea urchin sperm [98], consistent with earlier conceptual models [99]. This finding, exemplified by the planar swimming of sea urchin sperm, underscores the critical importance of precise spatiotemporal regulation in the switching between the active and inactive states of dyneins. Although the regulation of sliding in mammalian sperm is likely more complex, given that their swimming generally exhibits a three-dimensional pattern, this discovery lays the foundation for understanding how the coordination of sliding underlies local flagellum bending.

Over half a century of research on sliding mechanisms has led to a proposal that flagellar bending itself triggers oscillatory motion through a self-regulatory system governed by mechanosensitive responses [50,100]. Detailed observations, involving both chemical and mechanical manipulations, indicate that local microtubule curvature generated by axonemal dynein activity at one site can induce curvature at adjacent sites along the longitudinal axis of the axoneme, ultimately leading to the propagation of bending [101,102]. While conceptual models have been proposed to explain the coordinated activation and inactivation of dyneins, conclusive experimental evidence and solid theoretical demonstrations remain elusive [50,99].

A remarkable discovery regarding the mechano-responsive properties of sperm flagella was reported by Izawa and Shingyoji [103]. In their study, they used glass microneedles to apply mechanical deformation to demembranated, immotile sperm flagella, and found that the acute deformation could induce oscillatory movement even under very low ATP concentrations. Furthermore, this deformation triggered the formation of bend pairs, which subsequently propagated along the flagellum—a critical process for initiating the cyclical bending essential for flagellar motion. The mechanical strain at both ends of the flagellum enabled dynein molecules to generate oscillatory movements, highlighting strain-dependent regulation of dynein activity as a key factor in sustaining these oscillations. This idea aligns with a recent study demonstrating that exposing sperm encapsulated within a droplet to ultrasound as a mechanical stimulus significantly enhances sperm motility [104]. These findings imply structural and mechanical organization of dynein complexes could work as mechano-responsive units that underpin the oscillatory propagation of local bending.

4.2. Sperm flagellum as a mechanoresponsive system

Earlier observations and theories suggest that the sperm flagellum functions as a self-regulatory motile unit, capable of sensing and generating mechanical force mediated through inherent signaling systems. As is well known, ATP provides the energy required for the mechanical actions of sliding, with dynein powerstrokes through ATP hydrolysis. However, it is important to note that ATP distribution alone is unlikely to regulate the precise timing of these powerstrokes underlying sperm flagellar beatings because the spatiotemporal regulation necessary for the oscillatory propagation of microtubule-dynein sliding would require highly complex systems, which surpass current biophysical understanding. In this context, ATP can be regarded as a necessary, but not sufficient, factor for coordinated microtubule-dynein sliding, unless present at significantly high concentrations. Instead,

mechanical force may serve as the actual input stimulus for the sliding system, triggering dynein activation through conformational changes, akin to other mechanosensitive molecules [105,106].

The cellular system for sensing and generating mechanical forces is not unique to sperm but is observed in various other cell types as well. For example, epithelial cells, such as Madin-Darby Canine Kidney (MDCK) cells and Xenopus embryos, have a propensity to generate contractile forces in response to mechanical stretch [107]. The transition from force sensation to force generation involves mechanical stimuli at receptor molecules, which then trigger the extracellular signal-regulated kinase (ERK) MAP kinase signaling cascade, leading to actomyosin constriction [108–110]. Importantly, mechanical force alone is not sufficient to activate these cellular signaling systems; it must be combined with chemical factors [107], as is also the case with the sperm flagellum as discussed above. The timescale of the epithelial mechanoresponse (~minutes) is significantly slower than that of the sperm flagellar response (~milliseconds), suggesting distinct molecular modes of action and kinetics, despite similarities in their regulatory systems.

4.3. Mechano-chemical feedback in sperm collective

Given the mechanoresponsive properties of sperm flagella, one can hypothesize that in dense environments, sperm collectives establish a regulatory system where the motility of individual sperm not only influences their own flagella but also triggers cell signaling underpinning the flagellar beating in neighboring sperm through mechanical interactions (Figure 3A). As discussed in Section 2.2, the flagellar beating of a single sperm generates yaw, which exerts localized forces on adjacent sperm flagella through hydrodynamic effects, thereby activating the mechanosensitive microtubule-dynein sliding responsible for flagellar beating. This interaction, though still hypothetical, likely contributes to the robust maintenance of parallel swimming with synchronized flagellar beats in a cluster.

In a broader context, the assembly of cells, each equipped with a mechanoresponsive system, can give rise to emergent dynamic properties in multicellular tissues (Figure 3B). It has been shown that ERK-mediated mechanical feedback, through pulling and/or pushing by constituent cells within the epithelium, orchestrates spatio-temporal patterns of ERK activity, mechanical forces, and cell movement across various tissues [108,111–115]. Additionally, in motile bacterial collective swimming, stress-induced mobility enhancement through hydrodynamic interactions among bacteria is essential for explaining their fluidization and collective swimming [40], suggesting that this phenomenon may be a common feature in dense living matter.

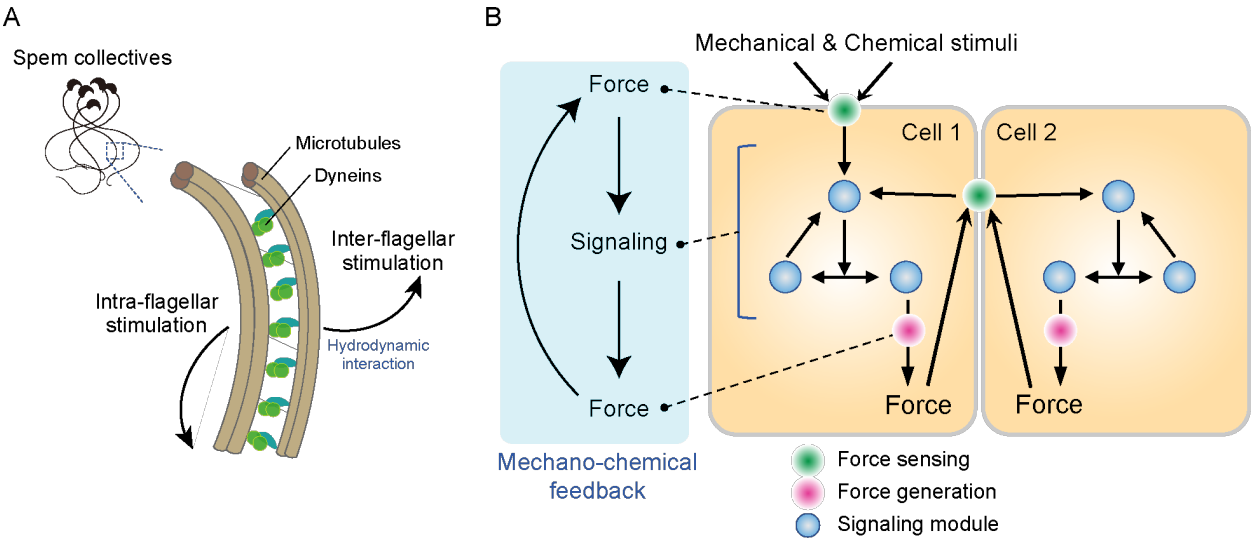

Figure 3: Schematics of mechano-chemical feedback in sperm collectives

A) Local microtubule-dynein sliding within a sperm flagellum can initiate beatings in both its own flagellum and neighboring flagella via hydrodynamic interactions.

B) General schematic of mechano-chemical feedback in multicellular systems.

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Competing interests

The authors declare no competing interests.

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