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Review

Flagellar ion channels of sperm: similarities and differences between species

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ABSTRACT

Motility and fertilization potential of mammalian sperm are regulated by ion homeostasis which in turn is under tight control of ion channels and transporters. Sperm intracellular pH, membrane voltage and calcium concentration ($[Ca^{2+}]_i$) are all important for sperm activity within the female reproductive tract. While all mammalian sperm are united in their goal to find and fertilize an egg, the molecular mechanisms they utilize for this purpose are diverse and differ between species especially on the level of ion channels. Recent direct recording from sperm cells of different species indicate the differences between rodent, non-human primate, ruminant, and human sperm on the basic levels of their ion channel regulation. In this review we summarize the current knowledge about ion channel diversity of the animal kingdom and concentrate our attention on flagellar ion channels of mammalian sperm.

1. Introduction

Spermatozoa are remarkable cells, which have fascinated researchers since their early discovery back in the 1677 by Leeuwenhoek [1]. Sperm cells are diverse not only in their morphology but also in terms of the molecular mechanisms that drive their physiology. A spermatozoon is a haploid cell that uses ATP-powered motility to deliver its genetic material to the ovum, depositing the paternal genome to restore diploidy, thus generating an embryo. Sperm cells are terminally differentiated and are thought to be transcriptionally and translationally silent, meaning that spermatozoa are largely unable to synthesize new mRNA or translate it into new polypeptides [2,3]. Mammalian sperm may look morphologically simple, with a single flagellum and head comprised of a condensed nucleus, redundant nuclear envelope and acrosomal vesicle. However, they are equipped with sophisticated molecular mechanisms that allow successful navigation in the female reproductive tract. In addition, mammalian sperm cells vary in size and shape of their head with rodents, such as mouse and rat having a hook-like appearance, and primates or ruminants sharing flat, oval shaped structures (Fig. 1). The molecular mechanisms that allow sperm to successfully navigate the female reproductive tract also vary among species [4,5]. To succeed, a spermatozoon must sense the environment and adapt its motility, which is controlled by ATP production and flagellar ion homeostasis. Sperm intracellular pH, membrane voltage and calcium concentration ($[Ca^{2+}]_i$) are regulated by ion channels and transporters and are vital for sperm survival and fertility [6–33]. In this review we summarize the current knowledge about well-studied sperm ion channels, such as the calcium channel CatSper, proton channel Hv1, potassium channels of the Slo family, as well as recently discovered ion channels of sperm. We also discuss the functional diversity of sperm ion channels among mammalian species(Fig. 2).

2. Sperm morphology

Motility originates from the flagellum that is subdivided into three functional parts: mitochondria containing midpiece, a

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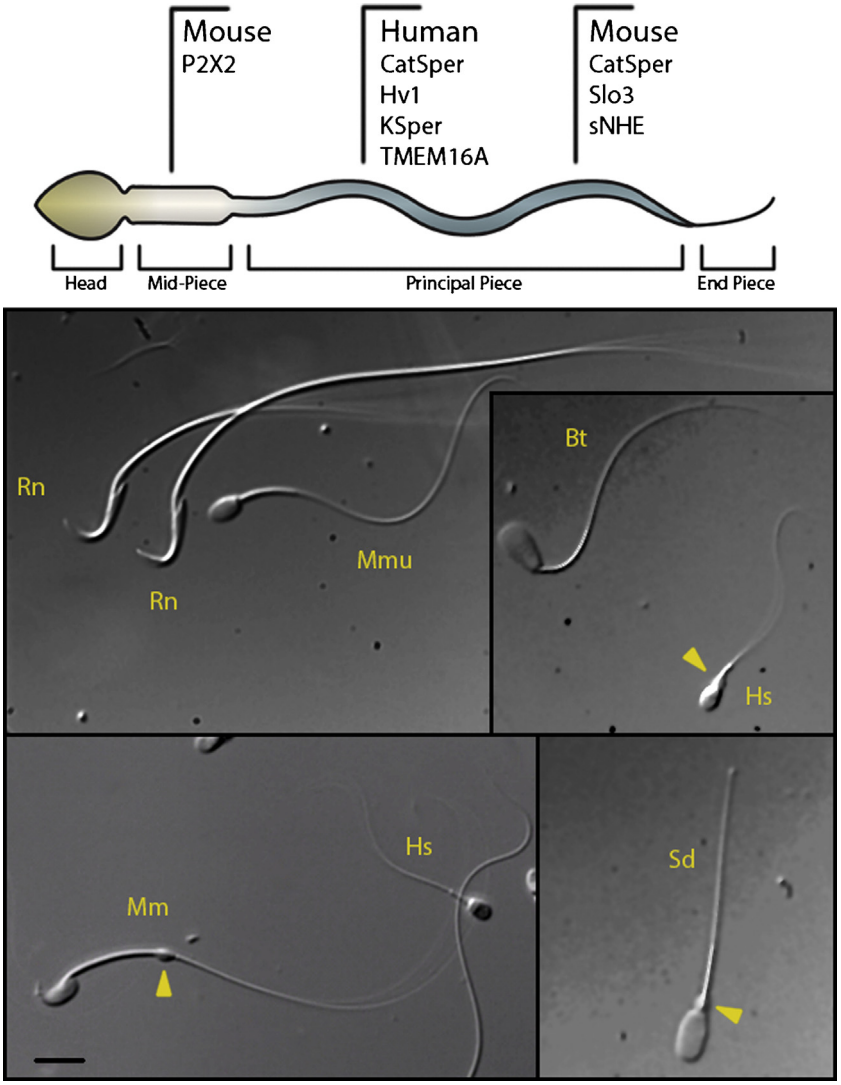

Fig. 1. Examples of sperm morphological and biochemical diversity. *Top panel:* schematic representation of spermatozoon with cellular compartments labeled and distribution of species-specific ion channels found within each section. *Bottom panel:* spermatozoa of different species are shown with cytoplasmic droplets indicated by yellow arrows. Shown are: human (Hs; *Homo sapiens*), mouse (Mm; *Mus musculus*), rat (Rn; *Rattus norvegicus*), rhesus macaque (Mmu; *Macaca mulatta*), boar (Sd; *sus scrofa domesticus*), and bull (Bt; *Bos taurus*) sperm cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

principal piece, and the endpiece (Fig. 1, top panel). The flagellum is in essence a motile cilium and is structurally similar in all sperm cells among multiple taxa in that they all have an axoneme with some modifications [34,35]. The flagellar plasma membrane is tightly attached to all underlying structures along the sperm body. This arrangement provides spermatozoa with a ridged structure to which membrane proteins can be tethered to the fibrous sheath [36] to ensure their strict compartmentalization [37]. Many sperm cells possess a single region of the sperm plasma membrane that is loosely attached to the rigid intracellular structures, a so-called cytoplasmic droplet (CD) [38,39]. The CD represents a remnant of the spermatid's cytoplasm and can be located around midpiece, or even more distally on the principal piece. The CD is shed from spermatozoa in certain species upon ejaculation; however, a small number of cells retains it [38,40]. Whether the presence of the CD is an indication of an immature spermatozoon is still questionable, a significant portion of fertile human sperm cells retain this structure. The CD has been proposed to serve as a reservoir for adaption to osmotic stress upon exposure to various environments [38,40–42]. It does however, serve another technical, but useful purpose: the CD allows for formation of tight, gigaohm contact (gigaseal) between sperm membrane and recording electrode which has made sperm patch clamp studies possible [16,43]. While many attempts have been made to study sperm ion channels indirectly [14,44–47], the real breakthrough came after introduction of whole-cell sperm patch clamp method [16,43] which has revolutionized the field of sperm ion channel research. In combination with various genetic models, the sperm patch clamp method provides a particularly useful tool for unbiased study of sperm ion channels of which calcium, potassium, proton, nonselective and various ligand-gated channels have been identified [19,21,48,49].

3. Calcium channels and hyperactivation

Calcium signaling is essential for all cell types, sperm being no exception. The maintenance and regulation of intracellular calcium concentration ($[Ca^{2+}]_i$), is therefore, of great importance and is carried out by proteins and co-factors that import, export and/or sequester calcium ions [50]. In spermatozoa, swimming behavior is controlled by rises in $[Ca^{2+}]_i$ that changes flagellar beat pattern through Ca^{2+} sensing proteins calaxins [51,52]. Calcium-bound calaxins inhibit the activity of dynein motors within the

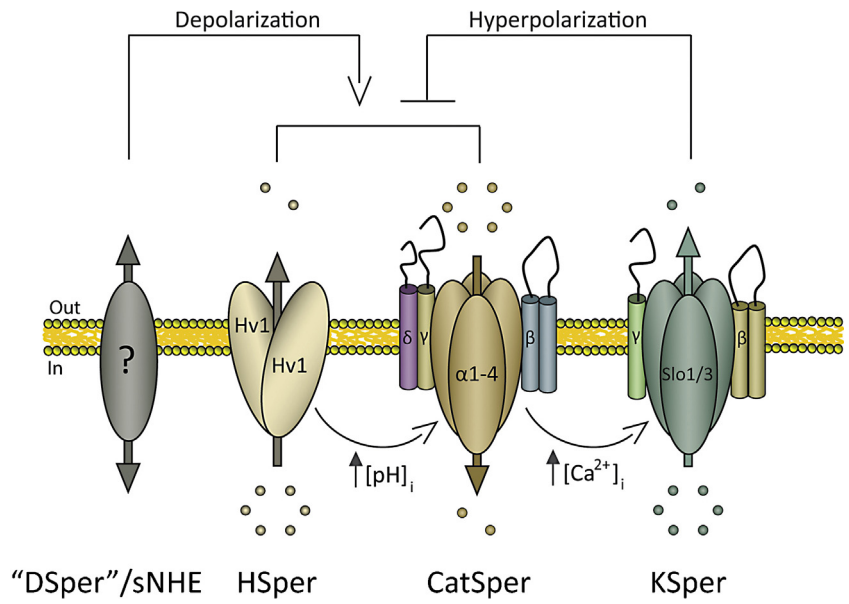

Fig. 2. Ion channel complexes of the sperm flagellum. Murine potassium channel KSper comprised of the Slo3 channel and associated gamma subunit LRRC52, as well as possible beta subunits. The identity of human KSper is still under debate but seems to share characteristics of the Slo family of ion channels. CatSper in both mouse and human share the same molecular composition consisting of at least 7 protein subunits to form a functional calcium permeable channel. Proton conductance via Hv1 channel is only detected in human sperm cells. Both CatSper and Hv1 require membrane depolarization in order to function. This could be achieved by inhibition of KSper. In addition, CatSper is activated by intracellular alkalinization that could be provided by Hv1 channel of human sperm. By moving protons out of the flagellum, Hv1 further hyperpolarizes the cell and provides negative feedback inhibiting both itself and CatSper activity. Therefore, the current model is missing fourth member, yet to be identified "depolarizing protein of sperm", aka "DSper". This hypothetical DSper could be activated by either membrane hyperpolarization as proposed for sNHE [112], or other mechanisms. DSper would provide positive net charge influx (such as an influx of Na^+) resulting in depolarization, consequent activation of Hv1/CatSper and sperm hyperactivation.

axoneme of the cell resulting in high-amplitude asymmetric flagellar bending [53]. This asymmetrical, whip-like bending of the flagellum, commonly referred to as hyperactivation, is essential for the ability of mammalian sperm to overcome the protective vestments of the oocyte. The propagation of a Ca^{2+}-induced wave produced from the opening of Ca^{2+} channels along the flagella is a necessary milestone in sperm maturation and makes hyperactivated motility possible [4,54,55]. Additionally, $[Ca^{2+}]_i$ plays a pivotal role in acrosome exocytosis [56–60]. Specific ion channels mediate the rise in $[Ca^{2+}]_i$ within sperm, and many candidates for the principle Ca^{2+} channel in sperm, including various voltage-gated Ca^{2+} channels, were proposed based primarily on studies using Ca^{2+} imaging, immunolabeling, and patch-clamp experiments from sperm precursors [61–63]. However, genetic ablation studies suggested that these candidate proteins were not functionally necessary for male fertility or some compensatory mechanism for their loss may be present [64–68]. The molecular identity of the first sperm specific ion channel required for male fertility in mice was determined in 2001 with the cloning of *CatSper1* [24]. The first member of the CatSper family of genes, CatSper1 is the pore forming α subunit of the Cation channel of Sperm (CatSper). Three other gene products later identified based on their similarity to CatSper1 also encode α subunits of the complex: CatSper2–4 [6,9,69,70]. Additionally, auxiliary subunits CatSperβ, γ, and δ have been shown to co-localize and associate with the CatSper complex, suggesting that CatSper is a heteromeric ion channel complex of at least 7 different subunits [71–73] (Fig. 2). Loss of any one member of the complex is detrimental to male fertility due to their interdependent protein expression in spermatozoa though CatSperβ and CatSperγ null mice have yet to be observed [9,19,22,24,49,74]. Humans with mutations within the *CatSper1* or *CatSper2* genes were also shown to be infertile revealing CatSper's importance as a general mechanism in mammalian fertility potential [6,75–80]. By combining mouse genetics with sperm patch clamp method CatSper activity was eventually directly recorded in 2006 and CatSper was established as the principal Ca^{2+} channel of mouse sperm [16]. Murine CatSper current is weakly voltage dependent with a slope factor (k) of 30, where true voltage-gated ion channel must have much steeper $k \sim 4$ [16]. This weak voltage dependency is thought to be the result of heterogeneity of the arginine and lysine composition of the putative voltage sensor domains within the CatSper α subunits. The characteristic CatSper current ($I_{CatSper}$) is lost in mice lacking any of the CatSper α subunits (CatSper1–4) or the accessory subunit CatSper δ and, as previously mentioned, are all infertile due to an inability of CatSper null spermatozoa to hyperactivate resulting in an inability of sperm to pass into the oviduct to reach the egg [9,10,16,21,22,24,71–73,81–83].

Human $I_{CatSper}$ was finally recorded in 2010 with the adaptation of the patch-clamp technique for human ejaculated spermatozoa [17,48]. Comparison of the CatSper current between the two species revealed fundamental differences in CatSper function and regulation. The voltage dependence of human CatSper is slightly steeper with $k \sim 20$. Furthermore, CatSper half-activation voltage ($V_{1/2}$) is strikingly different between the two species with $V_{1/2human} = +85$ mV at pH_i 7.4 and mouse $V_{1/2mouse} = +11$ mV under similar conditions. With this right shifted $V_{1/2}$, a significantly smaller fraction of human CatSper channels would be open under normal physiological conditions in comparison to mouse spermatozoa [17,18]. This suggests that the regulation of mouse and human CatSper are fundamentally different. Changing the intracellular pH from acidic to basic within the physiological range (6.0 to 7.4) induced a 7-fold increase of the murine CatSper current [16]. This coincided with a leftward shift in the GV curve of roughly −70 mV with the $V_{1/2}$ of mouse sperm, while human sperm $V_{1/2}$ was retained at +85 mV [16–18]. This suggests that at least for mouse, intracellular alkalinization is sufficient to open CatSper channels though for human CatSper the mere change in pH is not enough. This was unexpected as the N-termini of both CatSper1 proteins are highly enriched in histidines which were believed to account for the pH sensitivity of the channel. At the time of those studies the identity of the activation stimulus for human CatSper was still unclear.

The steroid hormone progesterone, produced and released by the cumulus cells surrounding the oocyte, was previously described to initiate an immediate increase in intracellular Ca^{2+} within human sperm cells [84,85]. The extracellular application of progesterone also rapidly triggers hyperactivated motility and initiation of the acrosome reaction [84–91]. Based on the fast activation rate, and fact that sperm cells are transcriptionally silent, it was believed that the nuclear progesterone receptor was not involved [85,88,92–95]. However, direct evidence of the instantaneous, and therefore non-genomic, effect of this steroid only came with the discovery that progesterone acts on human sperm through potentiation of the CatSper channel [18,27] with $EC_{50} \sim 7$ nM [18]. Progesterone shifts CatSper $V_{1/2}$ leftward in noncapacitated sperm cells with $V_{1/2noncap+Progesterone} = +53$ mV, however the shift is much more evident in capacitated cell with $V_{1/2cap+Progesterone} = +30$ mV [18]. Such observations were made in the absence of intracellular Ca^{2+}, ATP, GTP, or any other soluble second messengers suggesting that the progesterone effect observed acts through a receptor directly associated with the CatSper channel and not through G-proteins or protein kinases. Further evidence for CatSper as the source of progesterone-induced Ca^{2+} influx came from studies on a CatSper-deficient patient with a homozygous microdeletion in the *CatSper2* gene [6,79]. These CatSper-less spermatozoa didn't produce any progesterone- activated current, and even lacked basal CatSper activity [79]. While the molecular identity of sperm progesterone receptor is still unknown, the binding site for a cell impermeant analog of this steroid is accessible from the extracellular space [18]. Furthermore, evidence for non-genomic progesterone signaling in cells lacking CatSper channel expression [96], including progestin induced hypermotility in sperm cells of the Atlantic croaker [97], suggest that the initiation of membrane progesterone signaling is through a yet unidentified protein separate from the CatSper complex.

Together voltage, pH_i, and progesterone (human CatSper) work collectively to regulate Ca^{2+} influx into spermatozoa though other regulatory mechanisms may also exist. Further modulation of CatSper activity has been observed through direct recording of CatSper in the presence of prostaglandins [18,19]. Cholesterol efflux by serum albumin is a key component in sperm cell capacitation [85,98,99] and the influx of Ca^{2+} during this process has been linked directly to the presence of CatSper within sperm cells [100]. Environmental toxins including those known to disrupt the endocrine system have also been shown to induce intracellular Ca^{2+} elevation via the CatSper mechanism [101,102]. The regulation of $[Ca^{2+}]_i$ is critical for proper sperm function and thus compounds that directly modulate CatSper or effect $[Ca^{2+}]_i$ pose a genuine threat to sperm fertilization potential.

Despite advances in reproductive biology, roughly 80% of male infertility cases are idiopathic [103]. Point mutations within the *CatSper1* gene as well as a deletion of the *CatSper2* gene have been described in men with infertility [6,75,77,79]. Loss of expression of the *CatSper2* gene results in disruption of the entire complex as no appreciable CatSper current is observed in patient spermatozoa as well as a complete lack of detection of other CatSper complex members at the protein level [79]. Antibody recognition of CatSper is restricted to the principle piece of the sperm flagella and recent work by Chung et al. suggest that CatSper is localized to linearly arranged Ca^{2+} signaling domains along the length of the cellular structure [74]. Loss of CatSper expression as in *CatSper1-null* mice results in dysregulation of these Ca^{2+} signaling domains indicating that CatSper not only acts as a mechanism of Ca^{2+} entry in to the cell but also as an organizing, possibly anchoring, unit of intracellular signaling. The inability of CatSper deficient spermatozoa to hyperactivate, therefore, is attributable to the loss of the Ca^{2+} influx mechanism, but also the disruption of intracellular signaling pathways necessary for proper sperm function and maturation.

The CatSper channel is evolutionarily conserved in the genome of species from mammals to invertebrates such as sea urchins and sea squirts. Interestingly, the CatSper genes are lost in teleosts, amphibians, and birds [96]. At the time of writing this review, the only species with electrophysiologically confirmed CatSper current were human and mouse. Characterization of Ca^{2+} influx within sperm of different species has been attempted using optical methods including fluorescent Ca^{2+} dyes and motility studies. For instance, bovine [104] and equine [105] sperm are both sensitive to intracellular alkalization resulting in an influx of Ca^{2+} into the cell. Unpublished data from our lab has also shown that rat and primate sperm also have a CatSper-like current. From initial experimentation, rat CatSper shares a current density similar to that of human CatSper suggesting that there may be a stimulating factor that potentiates rat CatSper in the same way that progesterone stimulates the human channel. However, additional work is needed to confirm the molecular identity of the Ca^{2+} channel within these species and characterize the role of these channels in sperm function and during sperm maturation.

4. Proton channels and transporters

4.1. Mechanisms of proton extrusion from mammalian sperm

Intracellular pH is a key regulator of many sperm physiological processes including initiation of motility, capacitation, hyperactivation, chemotaxis and acrosome reaction. Even the basal sperm motility is pH-sensitive since dynein's ability to hydrolyze ATP and provide axonemal bending greatly increases with the rise of intracellular pH (pH_i). The motile sperm flagellum constantly generates intracellular protons via glycolysis, ATP hydrolysis and proton/calcium exchange [19]. The faster a flagellum moves, the more acidic it becomes. Babcock et al. [106,107] suggested that the mechanism for proton efflux from bovine sperm was *via* a voltage-gated proton channel, based on the fact that the sperm cytosol becomes alkaline upon membrane depolarization [106,107]. Later studies focused on the role of sperm-specific Na^+/H^+ (sNHE) and Cl^-/HCO_3^- exchangers as potential mechanisms for intracellular alkalization in rodent spermatozoa [32,108,109]. Due to the increase in external Na^+ upon ejaculation and its flagellar localization, the NHE was proposed as potential mechanism for sperm alkalization. Indeed, $sNHE^{-/-}$ mice were completely infertile due to impaired motility; however, they also had unexpectedly low expression of soluble adenylate cyclase (sACY) [110]. The fact that $sNHE^{-/-}$ sperm motility could be rescued by application of membrane permeable cAMP analogues has suggested that absence of sACY was perhaps the cause of the infertile phenotype [111]. An intriguing model was recently proposed [112] suggesting that sNHE may function as hyperpolarization activated proton extrusion mechanism since it possesses a putative voltage sensor [108,110,111,113].

In 2010, direct electrophysiological recordings of human sperm revealed a large voltage-activated outwardly rectifying H^+ current [17], aptly named HSper (H^+ channel of Sperm). HSper was shown to be highly H^+ selective, with strong outward conductance that was governed by H^+ gradients between the intracellular and extracellular environments as well as exhibiting an extreme sensitivity to Zn^{2+} [17]. Based on these unique biophysical properties, it was deduced that HSper closely resembles the voltage-gated channel Hv1 [114,115]. Interestingly, Hv1 was highly expressed in the flagellum indicating a potential role in the regulation of CatSper [17]. While Hv1 is ubiquitously expressed and plays an important role in a number of physiological processes such as the innate immune system, apoptosis, and cancer metastasis, the presence of Hv1 in

human spermatozoa was intriguing. Hv1 is not a true ion channel, but rather a strange mix between a transporter and an ion channel without the pore, that provides rapid movement of protons across a lipid bilayer via a voltage-gated mechanism [116–118].

4.2. Discovery of the voltage-gated proton channel Hv1

Voltage-gated proton channels were first proposed to play a role in bioluminescence in dinoflagellates [119]. The first electrophysiological recording of voltage-dependent proton current in snail neurons were reported by Thomas and Meech [120]. Later studies, primarily done by DeCoursey group [121–123], found similar currents in other cell types [124]. However, controversy in the field on whether proton channels were in fact molecular entities had been present since 1989 after studies from Byerly and Suen [125]. Such studies showed that the single channel conductance of this current under optimal conditions was almost 1000-fold smaller than that of other ion channels (~4 fA) [125], suggesting that these recordings could potentially be leak currents through the phospholipid bilayer. It was not until 2006, when two simultaneous publications: one from Dr. Clapham group [114], and another from Dr. Okamura group [115] put these doubts to rest. These groups identified proton channel gene in human [114], mouse and sea squirt genomes [115]. Molecular and electrophysiological studies of this channel have indicated four distinct features: (1) the channel is activated by membrane depolarization and increased $[pH]_i$, (2) is inhibited by micromolar concentrations of zinc, (3) is exquisitely H^+ selective and (4) has a uniquely unconventional structure that lacks a pore domain.

4.3. Hv1: Principles of proton permeation

The encoded protein is 273 amino acids in length and forms four transmembrane spanning segments (S1–S4), homologous in structure to the voltage-sensor of voltage-gated cation channels [114,115]. Unlike traditional voltage-gated ion channel, Hv1 does not possess a pore domain. Several models for the proton conductance pathway have been proposed [116,117,126]. The "water wire" model [117] proposes the movement of protons via Grotthus mechanism from the intracellular water vestibules to the extracellular vestibule formed by the transmembrane (TM) portions of the channel. Two vestibules are connected by a narrow bottleneck [117] where proton selectivity occurs via a highly conserved and unique aspartate moiety (Asp112 in human Hv1, [127]). Another model [116] suggests that Hv1 closed state favors electrostatic interactions between hydrophobic residues of TM segments and pull them together to form a hydrophobic plug. This hydrophobic layer is formed at the center of the voltage-sensing domain and prevents proton permeation. The latter occurs once the plug is removed by a voltage-dependent rearrangement of TMs and is followed by insertion of the protonatable residues in the center of the channel [116]. The recent crystal structure of the Hv1 in resting state sheds [118] light on the possible mechanism of H^+ permeation, suggesting that a combination of two hydrophobic plugs and a protonatable aspartate is required for proton movement. However, Hv1 structure in the open state is also required to completely understand this process.

Hv1 forms a functional dimer in the plasma membrane through a coil-coil interaction of the C-termini [128–130]; though, each Hv1 subunit can function independently as a channel. Mutations in Hv1 appear to be extremely rare with only one case of a single substitution mutation being reported in humans [131], which was assessed only in airway epithelial cells. Currently, there is no data regarding the effect of this mutation on fertility.

4.4. Human sperm Hv1

In order to fertilize an oocyte sperm cells have to undergo a process known as capacitation which comprises of a number of physiological changes including intracellular alkalization. In humans this is thought to be due to proton extrusion via Hv1 [17] (Fig. 2). At the time of ejaculation, spermatozoa are combined with the seminal plasma, which contains high concentrations of zinc (~2 mM) [48] and as such, directly inhibits Hv1 function by binding to two histidine residues that stabilize the channel in the closed state [132]. As sperm progress through the female reproductive tract, divalent zinc is chelated by proteins in the oviductal fluid resulting in gradual activation of Hv1 channel [48,133]. Electrophysiological data have shown that Hv1 is potentiated by micromolar concentrations of anandamide, an endogenous cannabinoid found in male and female reproductive fluid and thought to interact directly with Hv1 [17]. The physiological relevance of this effect has been the subject of controversy as the physiological concentration of anandamide in the male and female reproductive tract are much lower than the EC_{50} for Hv1 [134,135]. However, the cumulus cells surrounding the oocyte synthesize anandamide [134], therefore exposing sperm to higher concentrations during their transit through the cumulus mass [19,48]. Hv1 is also activated by capacitation [17], the process during which tyrosine phosphorylation of sperm proteins occurs. Although the exact cause of the Hv1 potentiation is still unclear it could be either due to potential phosphorylation of Hv1 or other protein modification. For example, in leukocytes it is known that phosphorylation of Thr29 enhances the activation of hHv1 [136]. It is therefore possible that Hv1 in human sperm may be regulated in a similar fashion. In contrast to human sperm, mouse spermatozoa do not possess Hv1, and, not surprisingly, Hv1-/- mice are fertile. The role of this channel in human fertility is still unclear and will remain so until an Hv1 defective patient or a representative animal model can be identified. Recent animal electrophysiological recordings from our lab assessing rat and bovine spermatozoa have indicated that these animals also do not possess an Hv1 current (an unpublished observation). Therefore, at present it would appear that humans are unique in this respect. It has yet to be determined whether sperm from other members of the primate family require Hv1 for proton extrusion. If this is the case, non-human primate models may prove to be an interesting model to assess the role of Hv1 in male fertility. In species that lack sperm Hv1, it is still unclear how intracellular pH is regulated in these cells, though sNHE remains an attractive mechanism. Unfortunately, as sNHE is suggested to be electro-neutral (no overall net charge), traditional electrophysiological techniques cannot be implemented to investigate if NHE is the alkalizing mechanism. It does, however, highlight unique species-specific differences in ion channels that regulate sperm physiology.

5. Potassium channels of sperm

Sperm membrane potential is vital for fertility, since both Hv1 and CatSper channels are voltage-dependent. In living cells, membrane potential is defined by the gradients of K^+, Na^+ and Cl^- with potassium channels playing a crucial role in its regulation. Noncapacitated epididymal murine spermatozoa are slightly depolarized at about −40 mV, however they hyperpolarize up to −60 mV upon capacitation [137]. This effect is attributed to potassium permeability and two members of the Slo family of potassium channels have been recently proposed to play a role in this process [21,25,26,29,31,33,138–141]. Slo3 (*Kcnu1*), a pH-sensitive, calcium-independent and weakly voltage-sensitive channel, has been identified as the principal potassium channel in murine sperm [25,26,29,31,33,141–143]. Slo3 was also sensitive to Ba^{2+}, quinine

and mibefradil that all reversibly inhibited murine Slo3 [29]. The identity and importance of the Slo3 channel for murine sperm physiology was confirmed by recordings from Slo3-deficient mice that also display severely reduced male fertility [25,31]. While Slo3 was also expected to form the potassium channel of human sperm, recent recording from ejaculated human spermatozoa indicates that in clear contrast to murine sperm, human sperm potassium current (KSper) is pH-independent and sensitive to $[Ca^{2+}]_i$ [20]. Furthermore, it could also be inhibited by progesterone [20,144]. From these biophysical properties as well as the pharmacological profile, human KSper seems to resemble the calcium-activated big conductance potassium channel Slo1 (*KCNMA1*) [20]. Recently other models were proposed suggesting that human capacitated spermatozoa possess a different type of Slo channel [145] or even a modified version of Slo3 that is calcium-sensitive and weakly pH-dependent [144], an unusual set of properties for this type of ion channel (Fig. 2). The reason why this is unconventional is that Slo channels are defined based on their gating properties, for example Slo3 is pH-gated, Slo2 is Na^+-gated and Slo1 is gated by Ca^{2+} [26,146–148]. Furthermore, the crystal structure of human Slo3 indicated the channel lacks a calcium-sensitive bowl [142]. One apparent difference is that both studies used slightly different human sperm cells: capacitated [144] versus noncapacitated cells [20], but another possible explanation could be that KSper consists of a heteromer of Slo1 and Slo3 subunits [145,149]. While Slo channels exist as tetrameric complexes of alpha subunits (Slo1 or Slo3) and auxiliary gamma subunits (LRCC26 or LRCC52), additional beta subunits can change their biophysical and pharmacological properties [140,150,151]. Uncovering the precise molecular identity of the human potassium channel remains an essential task for understanding the regulation of potassium homeostasis in human spermatozoa. As with the CatSper channel, human genetics may provide a useful tool to answer this question.

6. Nonselective and emerging ion channels

The sperm patch-clamp technique opens the door for unbiased search for other sperm ion channels, including ligand-gated and polymodal ion channels. Recently Navarro et al., reported the presence of an ATP-gated P2X2 ion channel that is cation-nonselective and originates from the midpiece of murine sperm cells [152]. *P2rx2*-deficient male mice are fertile and have normal sperm morphology and other sperm parameters, however their sperm lack I_{ATP} and fertility of *P2rx2*−/− males declines with frequent mating [152]. Human spermatozoa, however, do not show the same I_{ATP} which indicates that P2X2 ion channels might not be expressed in human sperm cells ([153] and unpublished data from our lab). Calcium-activated chloride channels (anoctamins) have been recently found in human sperm [154], but not in mouse [30], as well as aquaporins, water channels that are required for sperm osmoadaptation [155]. Several members of the transient receptor potential (TRP) ion channel family [156–158] were thought to function in mammalian sperm cells. These include TRPM8, TRPV1, TRPA1 and others [19]. However, mice deficient in TRPV1–4, TRPA1, and TRPM8 have no obvious defects in sperm morphology or male fertility [156,157]. Moreover, CatSper promiscuity toward high concentrations of exogenous activators, such as menthols, may account for the mistaken identity of TRPM8 in human spermatozoa [153]. However, it is possible that other species that do not have CatSper activity rely on different flagellar channels and therefore the role of TRP channels in sperm is yet to be determined.

7. Conclusions

On their route to the egg mammalian spermatozoa encounter multiple barriers: viscous mucus, the narrow lumen of the uterotubal junction, complex maze formed by the epithelial folds of the fallopian tubes, and finally the protective vestments of the egg. In order to overcome these barriers, the sperm cell must sense the cues released by the egg and change its swimming behavior. Sperm can achieve this by increasing the amplitude and driving force of their tail bending, changing their direction of movement, and releasing special enzymes to dissolve the egg's protective vestments. Such sperm responses depend upon electrical activity of the sperm ion channels that open in response to environmental cues within the female reproductive tract. This in turn changes conductance of the sperm plasma membrane and sperm behavior. Influx of calcium through the flagellar calcium channel CatSper results in the flagellar bending and hyperactivation of motility. CatSper, in turn is activated by intracellular alkalinization, provided by perhaps sNHE or Hv1 channel (in human sperm) and membrane depolarization, which results from KSper inhibition. The same compartmental flagellar localization of sperm ion channels provides fine-tuned regulation of sperm motility (Fig. 2). It is possible that many cases of idiopathic male infertility can be attributed to malfunctioning of sperm ion channels and their effector molecules. While these channels work in a concerted manner to regulate motility, the molecular repertoire and mode of regulation is divergent between sperm cells of different species. The sperm patch-clamp technique opens many possibilities to uncover regulation of sperm ion channels and provide an insight into the molecular machinery of these flagellated cells.

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