KSper, a pH-sensitive K+ current that controls sperm membrane potential

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Maternal mammalian spermatozoa are quiescent in the male reproductive tract. Upon ejaculation and during their transit through the female reproductive tract, they undergo changes that enable them to fertilize the egg. During this process of capacitation, they acquire progressive motility, develop hyperactivated motility, and are readied for the acrosome reaction. All of these processes are regulated by intracellular pH. In the female reproductive tract, the spermatozoan cytoplasm alkalinizes, which in turn activates a Ca2+ Selective current (I_{Ca, sper}) required for hyperactivated motility. Here, we show that alkalization also has a dramatic effect on membrane potential, producing a rapid hyperpolarization. This hyperpolarization is primarily mediated by a weakly outwardly rectifying K+ current (I_{K, sper}) originating from the principal piece of the sperm flagellum. Alkalization activates the pH-sensitive I_{K, sper}, setting the membrane potential to negative potentials where Ca2+ entry via I_{Ca, sper} is maximized. I_{K, sper} is one of two dominant ion currents of capacitated sperm cells.

Before fertilization, mammalian sperm must undergo a poorly understood process called capacitation (1). Capacitation refers to a series of biochemical and physiological changes in the sperm cell that enable it to reach and fertilize the egg. Among these changes is an increase in the intracellular pH (pH(I)) (2) and hyperpolarization of the sperm plasma membrane potential (V_{m}) (3, 4). Resting pH(I) of mammalian sperm is ~6.5 as determined by pH-sensitive fluorescent probes (2, 5–7). After in vitro capacitation in bicarbonate-containing media at pH 7.4, pH(I) increases ~0.3 units (2, 8, 9). When intracellular alkalization is prevented by glucose incubation, bovine sperm fail to capacitate (8, 10).

Measurements with voltage-sensitive fluorescent dyes indicate that noncapacitated murine sperm are relatively depolarized (approximately ~30 mV) and hyperpolarize to approximately ~60 mV during capacitation (4, 11, 12). Indirect measurements attribute the capacitation-associated hyperpolarization to an increase in K+ permeability (4) and a block of epithelial sodium channels (ENaCs) (13). Interestingly, addition of BaCl2 (a nonspecific K+ channel blocker) to the capacitating medium prevented the hyperpolarization and the acrosome reaction (12). Several K+ channels have been reported in mammalian sperm (ref. 14; see also reviews in refs. 15 and 16), but only recently have patch-clamp methods been developed that allow whole-sperm-cell currents to be recorded under voltage clamp (17). To date, the inward Ca2+ Selective current, I_{Ca, sper}, but not outward K+ Selective current, has been described in detail (17, 18). By using whole-spermatozoan current clamp, we find that pH(I) largely determines mouse sperm membrane potential. Under whole-sperm and whole-flagellum voltage clamp, a pH(I) Sensitive K+ current (I_{K, sper}) was identified that sets the epididymal spermatozoan membrane potential.

Results

pH Controls Sperm Resting Membrane Potential. Mouse sperm cells are long (~100 μm) and vary in width from 2 μm at the head to <0.5 μm at the tip of the tail. There also is a relatively tight constriction (~1 μm) between the head and midpiece. To establish that the patch-clamp pipette accesses the entire sperm cell, Lucifer yellow dye was loaded into the pipette (see ref. 17). Within 10 sec after gigaseal formation and break-in, the Lucifer yellow dye (molecular weight, 522) was seen throughout the spermatozoan midpiece, head, and principal piece (Fig. 1a). In current-clamp mode, the resting sperm membrane potential could then be measured. At pH 6.0 and in a bath of physiological saline (see Materials and Methods), the resting sperm membrane potential was ~0 mV at 1.5 min after break-in (Fig. 1b). Intracellular alkalization, induced by 5 mM NH4Cl in the bath, dramatically hyperpolarized the membrane potential to ~45 mV within a few seconds.

Reduction of Cl− ions in the bath solution (from 144 to 4 mM) and addition of the ENaC channel blocker amiloride (25 μM) did not prevent alkalization-induced hyperpolarization, suggesting that the hyperpolarization does not result from active Cl− or ENaC channels (Fig. 1b). By switching between current- and voltage-clamp modes during the experiment, the effect of intracellular alkalization on the ion currents was determined. NH4Cl-induced alkalization increased outward current >7-fold (Fig. 1c), which reversed upon NH4Cl washout. Decreasing extracellular Cl− ions from 144 to 4 mM (Fig. 1c) did not affect this potentiation of outward current, again excluding a significant role for Cl−- permeable channels. When the pipette was buffered to pH 6, alkalization induced by NH4Cl dramatically shifted sperm membrane potential from ~7 ± 1 to ~54 ± 1.6 mV. In contrast, when the pipette was pH 7.0, alkalization by NH4Cl induced only a ~5 mV shift, from ~54 ± 1.1 to ~58.6 ± 2 mV (Fig. 1d). Thus, pH(I) sets the sperm membrane potential primarily by modifying a K+ Conductance.

Properties of the Spermatozoan Potassium Current (I_{K, sper}). Immediately after break-in under voltage clamp, a constitutively active, weakly outwardly rectifying K+ current was recorded (symmetrical 160 mM [K+]) (Fig. 2a). To avoid contamination from monovalent currents via CatSper channels in nominal Ca2+ -free conditions (17) [supporting information (SI) Fig. 5], spermatozoa from CatSper1−/− mice were used in subsequent recordings (I_{K, sper} was unaffected by the absence of CatSper1 protein). In step depolarizations, I_{K, sper} exhibited no time or voltage dependence (Fig. 2b). I_{K, sper} was relatively K+ Selective; the reversal potential was near zero, suggesting that I_{K, sper} was a K+ Selective current.

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Abbreviations: pH(I), intracellular pH; ENaC, epithelial sodium channel; EIPA, 5-(N-ethyl-N-isopropyl)amiloride; 4-AP, 4-aminopyridine.

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potential was 1.2 ± 0.34 mV in 160 mM [K⁺]o and −72.5 ± 0.71 mV in 5 mM [K⁺]o (160 mM [K⁺]o; predicted E_K = −88 mV at 22°C) (Fig. 2c). P_{Na/P}\_K could not be determined because of a contaminating Na⁺ current (SI Fig. 6a) under bionic conditions (SI Fig. 6b) and K⁺ accumulation in the sperm flagellum (SI Fig. 6c) (see also ref. 17).

One advantage of spermatozoa is that they can be divided into two intact fragments (head + midpiece or midpiece + principal piece) and patch-clamped separately (17). I_{KSper} originated from the principal piece of the sperm flagellum as shown by recordings from head + midpiece and midpiece + principal piece fragments (Fig. 2e and f). Averaged outward currents of the two respective fragments were 11.9 ± 4.4 pA (n = 6; +100 mV) and 442.9 ± 32.7 pA (n = 4; −100 mV). In summary, I_{KSper} is a weakly outwardly rectifying K⁺ current originating from the principal piece of the sperm flagellum. Thus, I_{KSper} and I_{CatSper} are flagellar-specific currents.

**I_{KSper} Is Strongly Potentiated by Intracellular Alkalization.** Intracellular acidification inhibited, whereas alkalization significantly potentiated, I_{KSper} (Fig. 3). Average I_{KSper} increased ~8-fold when pH_i changed from 6.0 to 8.0 without affecting the overall shape of the I-V relationship (Fig. 3a and b). Intracellular alkalization induced by bath addition of 5 mM NH₄Cl strongly potentiated I_{KSper}. This potentiation was independent of [K⁺]o (Fig. 3b) and thus unlikely to be caused by increased permeation of NH₄Cl through I_{KSper} channels.

Extracellular pH also affected the amplitude of I_{KSper}. On average, currents were smaller at acidic pH_i and larger at alkaline pH_i (Fig. 7). This effect was likely because of changing pH_i rather than direct effects by external protons; as long as the internal buffer capacity was sufficient to control pH_i, pH_o had little effect on I_{KSper}.

I_{KSper} was not affected by 2 mM membrane-permeant cAMP and cGMP analogs (SI Fig. 8), by increasing [Ca²⁺]o to 2 mM or [Ca²⁺]i (up to 10 μM), or by changes in bath osmolarity (230–350 mOsm) (SI Fig. 9). The response of I_{KSper} to pH was identical in WT and CatSper⁻⁻ sperm cells. Hence, I_{KSper} is a pH_i-sensitive channel that is activated in vivo by intracellular alkalization.

**I_{KSper} Antagonists and Their Effect on Sperm Membrane Potential.** Quinine (500 μM), an antagonist of many K⁺ channels, reversibly inhibited I_{KSper} by 88% (at +100 mV) (Fig. 4e). Clofazin (50 μM), a HERG (Kv11.1), Kc1.5, and TASK2 (Kp2.1) blocker irreversibly reduced I_{KSper} by 92%. Unexpectedly, 50 μM 5-(N-ethyl-N-isopropyl)amiloride (EIPA) (an amiloride analog and Na⁺/H⁺ exchanger antagonist) reversibly inhibited I_{KSper} by 72%, whereas the ENaC antagonist, amiloride, had no effect (at
up to 1 mM). Mibebradil (5 μM), an antagonist of CaV3 (T-type), two-pore K+ channels (K₂p), and KV1.5, reversibly reduced \( I_{K^{\text{Sper}}} \) by 69%. In contrast, 2 mM BaCl₂, 10 mM tetraethylammonium-Cl, and 200 μM CdCl₂ (an inhibitor of Kᵥ4.3) minimally inhibited \( I_{K^{\text{Sper}}} \) (<2%). Ba²⁺ inhibited \( I_{K^{\text{Sper}}} \) only minimally in symmetrical 160 mM [K⁺], perhaps because K⁺ is harder to displace from the pore under these conditions. However, when Na⁺ is the dominant extracellular cation, Ba²⁺-blocked \( I_{K^{\text{Sper}}} \) by ≈50% (SI Fig. 10). 4-Aminopyridine (4-AP), a nonspecific K₅ channel antagonist, enhanced \( I_{K^{\text{Sper}}} \) as well as \( I_{\text{CatSper}} \), but this was most likely a consequence of its known alkalinization of cells (pKᵢ, 9.2; at physiological pH, ≈1% is present as the uncharged membrane-permeant pyridine) (SI Fig. 11) (19). Thus, quinine, clofilium, EIPA, 4-AP, Ba²⁺, and mibebradil were tested for their effects on sperm membrane potential under current clamp.

Sperm membrane potential appears to be largely determined by pH-sensitive \( I_{K^{\text{Sper}}} \). At resting pH₂ (≈6.0–6.5), even very small currents can shift the membrane potential because the

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**Fig. 2.** Endogenous sperm cell K⁺ current (\( I_{K^{\text{Sper}}} \)). (a) Weakly outwardly rectifying K⁺ current from a WT spermatozoa measured in symmetrical 160 mM [K⁺] (pH 8.0) in response to a 1-sec ramp protocol from −100 to +100 mV (holding potential, HP, 0 mV). (b) \( I_{K^{\text{Sper}}} \) of CatSper¹⁻ spermatozoa in response to voltage steps. Whole-cell currents elicited by 1-sec steps from HP of 0 mV to test potentials between −100 and +100 mV (Δ = −20 mV, 5-sec intervals; pH 8.0). (c) Reversal potentials of a CatSper¹⁻ spermatozoa were measured in response to changing [K⁺], (pH 8.0) from 160 mM (black trace; \( E_{rev} = 1.2 ± 0.34 \text{ mV}; \ n = 30 \)) to 5 mM (red trace; \( E_{rev} = −72.5 ± 0.71 \text{ mV}; \ n = 21 \); predicted \( E_{K} = −87 \text{ mV} \)). Green trace, 0 mM [K⁺] (0). Head plus midpiece sperm fragment loaded with Lucifer yellow dye (Lower). Differential interference contrast (DIC) image (Upper). (Scale bar, 10 μm.) (d) Midpiece + principal piece sperm fragment loaded with Lucifer yellow dye (Lower). DIC image (Upper). (Scale bar, 10 μm.) (e) Current in CatSper¹⁻ spermatozoa in response to a 1-sec voltage-clamp ramp from −100 to +100 mV from the head + midpiece (red) and from the midpiece + principal piece (black; pH 8.0).

**Fig. 3.** Intracellular alkalinization strongly potentiates \( I_{K^{\text{Sper}}} \). (a) Steady-state whole-cell currents of CatSper¹⁻ spermatozoa at pH 6.0 (red), 7.0 (blue), and 8.0 (black). (b) Initial current (red) of CatSper¹⁻ spermatozoa in symmetrical 160 mM [K⁺] at pH 6.0. Addition of 5 mM NH₄Cl to the bath alkalinized the cytoplasm and induced \( I_{K^{\text{Sper}}} \) (black). Alkalinization-induced \( I_{K^{\text{Sper}}} \) in 5 mM (purple) or 80 mM (green) [K⁺]. (c) Average \( I_{K^{\text{Sper}}} \) amplitudes at −100 and +100 mV at varying pH₆. HP, holding potential.
spermatozoan has an exceptionally large input resistance ($R_m > 5 \ G\Omega$; $C_m \approx 2.5 \ pF$). As expected, quinine transiently depolarized sperm membrane potential, whereas clofilium did so reversibly (pH 7.0) (Fig. 4b). EIPA depolarized the sperm cell membrane potential (pH 7.0) (Fig. 4c) and blocked the hyperpolarization induced by NH$_4$Cl and 4-AP (pH 6.0) (Fig. 4d). Interestingly, Ba$^{2+}$, which weakly blocks $I_{\text{CatSper}}$, depolarized sperm cells (pH 7.0) (Fig. 4c and SI Fig. 12a) and blocked NH$_4$Cl-induced hyperpolarization (SI Fig. 12b). Because Ba$^{2+}$ permeates CatSper and potentially could induce depolarization, the $I_{\text{CatSper}}$ blocker Ni$^{2+}$ (300 µM) was added together with Ba$^{2+}$ in some experiments. $I_{\text{CatSper}}$ block did not affect Ba$^{2+}$-induced depolarization, suggesting that this effect is mainly through its block of $I_{\text{CatSper}}$. We also tested the effect of [K$^+$]$_i$ on membrane potential. Addition of 5 mM KCl to the bath induced a small depolarization ($\approx 3 \ mV$) (Fig. 1b), whereas 150 mM [K$^+$]$_i$, depolarized the membrane by $\approx 60 \ mV$ (SI Fig. 12a). In summary, antagonists that reduce $I_{\text{CatSper}}$ also dramatically depolarize sperm $V_m$.

Discussion

Our results show that pH$_i$ has a dramatic effect on sperm membrane potential, with alkalization producing a rapid hyperpolarization. This hyperpolarization is primarily mediated by an endogenous weakly outwardly rectifying, pH$_i$-sensitive K$^+$ current ($I_{\text{CatSper}}$) originating from the principal piece of the sperm flagellum. Together with the effects of $I_{\text{CatSper}}$ antagonists on sperm membrane potential and pH$_i$ sensitivity, we conclude that $I_{\text{CatSper}}$ is the dominant hyperpolarizing conductance within the physiological range and thus largely sets spermatozoan resting membrane potential. Sperm intracellular pH$_i$ roughly follows pH$_s$ (20). As sperm travel from the vagina (pH $\approx 5$) to the cervical mucus (pH $\approx 8$), they undergo intracellular alkalization. Consequently, the sperm’s membrane potential hyperpolarizes as pH$_i$ increases in the female reproductive tract.

In this and previous work, we characterized two of the primary ion conductances of epididymal mouse spermatozoa, $I_{\text{CatSper}}$ and $I_{\text{CatSper}}$. Both $I_{\text{CatSper}}$ and $I_{\text{CatSper}}$ are sensitive to pH$_i$ in the physiological range and originate specifically from the principal piece of the sperm flagellum. Alkalization hyperpolarizes sperm by activating $I_{\text{CatSper}}$ and at the same time dramatically shifts the activation potential of $I_{\text{CatSper}}$ to the hyperpolarized range. Because at alkaline pH $I_{\text{CatSper}}$ is active, hyperpolarization will increase the driving force for Ca$^{2+}$ entry ($E_{Ca} > +150 \ mV$). The primary effect of this simple change is to increase intracellular [Ca$^{2+}$] and induce hypertactivated motility. However, currents that might be induced by unknown native agonists, and the uncharacterized Na$^+$-carrying current, could mediate other effects on sperm membrane potential and physiology.

The most likely gene responsible for $I_{\text{CatSper}}$ is mSlo3 (21). Like the CatSper channels, mSlo3 appears specific to testes and has not been functionally expressed in mammalian cell lines. mSlo3 expression in Xenopus oocytes yielded measurable currents, and...
these currents were activated by intracellular alkalinization (21, 22). Like \( I_{\text{KSper}} \), *Xenopus* oocyte-expressed mSlo3 is also weakly voltage-sensitive (~16 mV/e-fold), has relaxed \( K^+ \)-selectivity, and is insensitive to \( [\text{Ca}^{2+}]_\text{c} \) and external tetraethylammonium. Available anti-mSlo3 antibodies were not specific enough to allow immunocytochemical identification or localization. Further evidence that \( I_{\text{KSper}} \) is mediated by mSlo3 will require deletion of this gene in mice.

### Materials and Methods

Whole-cell recordings were made on sperm cells from the corpus epididymis from mice 3–8 months of age, as reported (17). The standard bath solution (HS) contained the following: 135 mM NaCl, 5 mM KCl, 2 mM CaCl\(_2\), 1 mM MgSO\(_4\), 20 mM Hepes, 5 mM glucose, 10 mM lactic acid, 1 mM Na pyruvate, pH 7.4 (with NaOH). After break-in, the access resistance was 25–80 MΩ. The standard pipette solution was as follows: 115 mM K-methanesulfonate (K-MeSO\(_4\)), 5 mM KCl, 10 mM K\(_2\)-BAPTA, 20 mM Hepes, and 20 mM Mes (pH 8.0 with Trizma base or pH 6.0 with methanesulfonic acid). A weak pH-buffered pipette solution was used in some experiments and contained the following: 130 mM K-MeSO\(_4\), 5 mM KCl, 1 mM K\(_2\)-BAPTA, 5 mM K-Hepes, and 5 mM K-Mes (pH 6.0). In symmetrical 160 mM \([K^+]_\text{c}\) experiments, the bath solution was 150 mM K-MeSO\(_4\), 10 mM K-Hepes, and 10 mM Mes (pH 7.4). The bath solution for biionic experiments contained the following: 160 mM Na-MeSO\(_4\), 5 mM K-MeSO\(_4\), 10 mM Hepes, 10 mM Mes (pH 7.4); for \( E_{\text{rev}} \) measurements, it was as follows: 5 mM K-MeSO\(_4\), 170 mM Hepes (pH 7.4). The pipette solution for current-clamp experiments contained the following: 130 mM K-MeSO\(_4\), 5 mM KCl, 15 mM NaCl, 3 mM MgATP, 0.5 mM Na\(_3\)GTP, 1 mM K\(_2\)-BAPTA, 5 mM K-Hepes, and 5 mM K-Mes (pH 6.0 or 7.0). The low Cl\(^-\) solution was as follows: 150 mM Na-MeSO\(_4\), 5 mM K-MeSO\(_4\), 2 mM CaCl\(_2\), 10 mM Hepes, and 10 mM Mes, pH 7.4. For tests of osmolarity, the bath solution was as follows: 110 mM K-MeSO\(_4\), 10 mM Hepes, and 10 mM Mes (230 mM osm; pH 7.4), with mannitol being added to increase osmolarity.

Headless and tailless sperm cells (17) were prepared by incubating the sperm cell suspension in the presence of 0.2 mg/ml trypsin at 37°C for <5 min, followed by gentle trituration. All experiments were performed at 22–24°C. EIPA, amiloride hydrochloride hydrate, and quinine were dissolved in DMSO (final <0.1%). 4-AP, mibebradil, and clofilium were water-soluble.

Antagonists were diluted in HS solution to their final concentration and then perfused into the recording chamber. All currents were recorded by using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA), acquired with Clampex (pClamp; Molecular Devices), and analyzed with Origin software (OriginLab, Northampton, MA). Signals were low-pass-filtered at 2 kHz and sampled at 10 kHz. Data are given as mean ± SEM.

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SUPPORTING INFORMATION FIGURES

Supporting Information Figure 5. Separation of $I_{\text{CatSper}}$ and $I_{\text{KSper}}$ in wt sperm cells.

A. Addition of 2 mM Ca$^{2+}$ (red trace) slightly reduced the net inward current by reduction of monovalent $I_{\text{CatSper}}$.

B. $I_{\text{KSper}}$ (black trace) in symmetrical 160 mM [K$^+$]. Under bi-ionic conditions (160 mM [Na$^+$]$_o$, 5 mM [K$^+$]$_o$), contamination by monovalent $I_{\text{CatSper}}$ becomes apparent (pink trace; compare with SI Fig 6 B, orange trace).

Supporting Information Figure 6. Unidentified Na$^+$ current; K$^+$ accumulation in CatSper$^{1/-}$ spermatozoon.

A. Large Na$^+$ inward current recorded at very hyperpolarized potentials (-180 mV) in CatSper$^{1/-}$ sperm cell. This current has not been characterized in detail, and is temporarily named $I_{\text{NaSper}}$. Dashed line indicates 0 current level.

B. Reversal potential under bi-ionic conditions (orange trace; 160 mM [Na$^+$]$_o$, 5 mM [K$^+$]$_o$) was determined by $I_{\text{KSper}}$ and $I_{\text{NaSper}}$. Black trace represents $I_{\text{KSper}}$ in symmetrical 160 mM [K$^+$]. The blue trace is the net current composed of both $I_{\text{NaSper}}$ and $I_{\text{KSper}}$ (160 mM [Na$^+$]$_o$).

C. In symmetrical 160 mM N-methyl D-glucamine (NMDG; a large impermeant cation), no currents were recorded from CatSper$^{1/-}$ spermatozoa (red trace). However, when NMDG in the bath solution was replaced by K$^+$ (black trace), $I_{\text{KSper}}$ was active, and K$^+$ accumulated in the sperm flagellum. The large
outward current (black trace) is thus due to outward $K^+$ flux via the KSper conductance.

**Supporting Information Figure 7. Extracellular acidification reduces $I_{KSper}$**
A. $I_{KSper}$ at different $pH_o$ in symmetrical 160 mM [K$^+$] ($pH_i$ 7.0).
B. Average $I_{KSper}$ amplitudes at $-100$ and $+100$ mV at varying $pH_o$.

**Supporting Figure 8. Effects of membrane-permeant intracellular cyclic nucleotides on $I_{KSper}$**
A. Addition of membrane-permeant cyclic nucleotides (2 mM 8-Br-cAMP, red trace; 2 mM 8-Br-cGMP, green trace) does not alter $I_{KSper}$. Control: black trace. Representative traces from 7 recordings under each condition.
B. sp-8pCPT-cGMP (20 $\mu$M, pink trace). Representative trace from 5 recordings.

**Supporting Information Figure 9. $I_{KSper}$ is insensitive to changes in osmolarity.**
A. Change of bath solution from isosmotic (305 mOsm; black trace) to hyperosmotic (350 mOsm; red trace) or hypo-osmotic (250 mOsm; green trace) solutions had no effect on $I_{KSper}$ at $pH_i$ 8.0. Representative traces from 3 sets of recordings.
B. $I_{KSper}$ at $pH_i$ 6.0 was not affected by an extremely hypo-osmotic solution (230 mOsm; red trace). Representative of 3 similar recordings.
Supporting Information Figure 10. Barium block of $I_{KSper}$.

A. $I_{KSper}$ in response to a voltage ramp (wt; -100 mV to +100 mV; pH$_{i}$ 7.0) in Na$^+$ solution (HS; black trace) and in HS containing 2 mM BaCl$_2$ (red trace; representative of 3 experiments, avg. block = 53%).

B. $I_{KSper}$ in response to a voltage ramp (wt; -100 mV to +100 mV; pH$_{i}$ 6.0) in Na$^+$ solution (HS; blue trace), in HS containing 1 mM NH$_4$Cl (black trace), and in HS containing 1 mM NH$_4$Cl and 2 mM BaCl$_2$ (red trace; representative of 4 experiments, avg. block = 48%).

Supporting Information Figure 11. 4-AP increased $I_{KSper}$ via intracellular alkalinization.

A. $I_{KSper}$ was reversibly potentiated by [4-AP]$_o$ in pH$_{i}$ = 6.0.

B. $I_{KSper}$ potentiation by [4-AP]$_o$ was absent when pH$_{i}$ = 8.0.

C. $I_{KSper}$ potentiation by [4-AP]$_o$ was reduced when [pH buffer]$_i$ was increased from 5 to 20 mM.

D. In symmetrical pH 7, $I_{KSper}$ was potentiated by [4-AP]$_o$.

E. $I_{KSper}$ amplitude was increased by [4-AP]$_o$ at constant 4 mM [4-AP]$_i$, with low [H$^+$] buffer capacity (5 mM HEPES/MES). This implies that entry of 4-AP into the sperm cell is able to alkalinize the cytoplasmic compartment.

Supporting Information Figure 12. Membrane potential is sensitive to [K]$_o$. 
A. Spermatozoon membrane potential (wt; pH\(_i\) 7.0) was reversibly depolarized by 2 mM BaCl\(_2\), 5 mM CsCl, or 160 mM K-MeSO\(_3\). Representative trace from 6 independent experiments.

B. NH\(_4\)Cl-induced hyperpolarization was reversed by the addition of 2 mM BaCl\(_2\) and 5 mM CsCl. Representative trace from 3 independent experiments (pH\(_i\) = 6.0).
SI Fig. 5
SI Fig. 6
**A**

Catsper1−/−

-100 mV 100 mV

1 s

160 K-MeSO₃ pH 7.4
160 K-MeSO₃ pH 8.0
160 K-MeSO₃ pH 7.0
160 K-MeSO₃ pH 6.0
160 K-MeSO₃ pH 5.0
160 K-MeSO₃ pH 4.0

**B**

![Graph showing current (I) vs. voltage (Vm) for different pH values](SI Fig. 7)
SI Fig. 8
SI Fig. 9
A

wt

HP 0 mV

-100 mV

1 s

100 mV

1 K_4-BAPTA
3 Mg-ATP
5 K-HEPES
5 K-MES
and
130 K-MeSO_3
5 KCl
15 NaCl
pH 7.0

HS

HS + 2 BaCl_2

I (pA)

Vm (mV)

-180 -60 -50

-60 -50

-100

B

wt

HP 0 mV

-100 mV

1 s

100 mV

1 K_4-BAPTA
3 Mg-ATP
5 K-HEPES
5 K-MES
and
130 K-MeSO_3
5 KCl
15 NaCl
pH 6.0

HS

HS + 1 NH_4Cl

HS + 1 NH_4Cl + 2 BaCl_2

I (pA)

Vm (mV)

-180 -60 -50

-60 -50

-100

SI Fig. 10
SI Fig. 11
SI Fig. 12