NIH Public Access
Author Manuscript
Dev Biol. Author manuscript; available in PMC 2008 March 1.

Published in final edited form as:
â*Dev Biol*. 2007 March 1; 303(1): 214â221.

Contributions of Extracellular and Intracellular Ca^{2+} to Regulation of Sperm Motility: Release of Intracellular Stores Can Hyperactivate CatSper1 and CatSper2 Null Sperm

Becky Marquez, **George Ignotz**, and **Susan S. Suarez**[1]
Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853

Abstract

In order to fertilize, mammalian sperm must hyperactivate. Hyperactivation is triggered by increased flagellar Ca^{2+}, which switches flagellar beating from a symmetrical to an asymmetrical pattern by increasing bending to one side. Thimerosal, which releases Ca^{2+} from internal stores, induced hyperactivation in mouse sperm within seconds, even when extracellular Ca^{2+} was buffered with BAPTA to approximately 30 nM. In sperm from CatSper1 or CatSper2 null mice, which lack functional flagellar alkaline-activated calcium currents, 50 ÎM thimerosal raised the flagellar bend amplitudes from abnormally low levels to normal prehyperactivated levels and, in 20â40% of sperm, induced hyperactivation. Addition of 1 mM Ni^{2+} diminished the response. This suggests that intracellular Ca^{2+} is abnormally low in the null sperm flagella. When intracellular Ca^{2+} was reduced by BAPTA-AM in wild-type sperm, they exhibited flagellar beat patterns more closely resembling those of null sperm. Altogether, these results indicate that extracellular Ca^{2+} is required to supplement store-released Ca^{2+} to produce maximal and sustained hyperactivation and that CatSper1 and CatSper2 are key elements of the major Ca^{2+} entry pathways that support not only hyperactivated motility but possibly also normal prehyperactivated motility.

Keywords

sperm; sperm motility; flagellum; calcium; calcium channel; calcium store; CatSper

INTRODUCTION

Ca^{2+} signaling in sperm is critical for fertilization. Ca^{2+} uptake (Handrow et al., 1989) is associated with capacitation, a process whereby mammalian sperm gain the capacity to undergo the acrosome reaction and fertilize oocytes (Yanagimachi, 1994). Activation of motility occurs when sperm are released from the cauda epididymidis. Activated sperm swim in linear trajectories generated by moderate amplitude symmetrical flagellar beating (Suarez et al., 1983). Elevation of flagellar Ca^{2+} during capacitation induces hyperactivation (Suarez and Dai, 1995;Ho and Suarez, 2001) by increasing the amplitude of the principal flagellar bend, which produces asymmetrical beating (Ho et al., 2002). Hyperactivated sperm exhibit frequent changes in direction that may enable them to navigate the tortuous lumen of the oviduct to reach the oocyte. During fertilization, the high amplitude flagellar bends of hyperactivation

[1]To whom correspondence should be addressed. Telephone: (607) 253-3589. Fax: (607) 253-3541. E-mail: sss7@cornell.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

are required by sperm to penetrate the oocyte zona pellucida (Stauss et al., 1995;Quill et al., 2003).

The mechanisms that trigger the rise of intracellular Ca^{2+} to initiate and maintain hyperactivation are not completely understood, but increased Ca^{2+} entry via plasma membrane channels is known to play a key role. Voltage-gated Ca^{2+} channels (Trevino et al., 2004;Wennemuth et al., 2000), cyclic-nucleotide gated Ca^{2+} channels (Wiesner et al., 1998), and canonical transient receptor potential (TRPC) channels (Trevino et al., 2001;Castellano et al., 2002) have been localized to the flagellum. However, only sperm-specific proteins CatSper1 (Ren et al., 2001) and CatSper2 (Quill et al., 2003) have been shown to be required for male fertility. These CatSper proteins are alkaline-sensitive voltage-gated Ca^{2+} channels that are located on the principal piece of the flagellum (Ren et al., 2001;Quill et al., 2001;Kirichok et al., 2006). Sperm deficient in CatSper1 or CatSper2 lack depolarization-evoked Ca^{2+} entry (Carlson et al., 2003;Carlson et al., 2005). Although it has not been demonstrated that CatSper1 and CatSper2 form a single channel, disruption of either gene prevents the expression of the other protein in the flagellum (Carlson et al., 2005). Sperm lacking CatSper show abnormally high flagellar beat frequency and low bend amplitude, as well as a failure to hyperactivate during capacitation (Carlson et al., 2003;Quill et al., 2003;Carlson et al., 2005).

There is also evidence for a mechanism in which release of Ca^{2+} from an IP_3-gated internal store at the base of the flagellum initiates hyperactivation (Ho and Suarez, 2001). IP_3 receptors have been localized to a portion of the redundant nuclear envelope on the side of the flagellum which increases bend amplitude in response to Ca^{2+} (Ho and Suarez, 2003). Because each flagellar bend originates at the base of the flagellum and is propagated down to the principal piece, it is therefore proposed that the store provides the initial Ca^{2+} signal, which somehow stimulates influx through CatSper channels in the principal piece.

The purpose of this study was to investigate the contributions of extracellular and intracellular Ca^{2+} to the initiation and maintenance of hyperactivated motility. Using CatSper1 and CatSper2 null sperm, we investigated whether release of Ca^{2+} from an internal store can initiate hyperactivation and if an extracellular Ca^{2+} source is required to sustain hyperactivation.

MATERIALS AND METHODS

Chemicals and Medium

All chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO) with the following exceptions. HEPES, BSA, and 1,2-bis(2-aminophenoxy)ethane-N,N,Nâ,Nâ-tetraacetic acid acetoxymethyl ester (BAPTA AM) were obtained from Calbiochem Corporation (La Jolla, CA). Molecular Probes (Eugene, OR) provided fluo3 AM and BAPTA.

The medium consisted of 110 mM NaCl, 2.68 mM KCl, 0.36 mM NaH_2PO_4, 25 mM $NaHCO_3$, 25 mM HEPES, 2.4 mM $CaCl_2$, 0.49 mM $MgCl_2$, 5.56 mM glucose, 1 mM pyruvate, 0.006% Na penicillin G, and 20 mg/ml BSA (pH 7.4, 290â300 mOsm/kg) (Suarez and Osman, 1987).

Animals

CatSper1 and CatSper2 null mice were provided by Drs. Timothy Quill and David Garbers (UT Southwestern) and were derived as previously described (Carlson et al., 2003;Ren et al., 2001;Quill et al., 2003;Quill et al., 2001). Males heterozygous (+/â) for *CATSPER1* or *CATSPER2* were used as controls in experiments involving males homozygous (â/â) for the disrupted genes, respectively, and wild-type (C57BL/6) males were used when testing the effects of BAPTA or BAPTA AM on motility.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Immunoblotting

Cauda epididymides were recovered from three male mice each of genotypes *CATSPER2* +/+, +/â, and â/â, placed in 1 ml warmed (37ÂC) PBS in a small culture dish, and nicked at several sites with small scissors to disperse sperm. After 15 min incubation, tissue was removed and the sperm-containing supernatants were transferred to eppendorf tubes. An aliquot of each was removed for hemacytometer counting, and sperm were pelleted by centrifugation. Sperm pellets were suspended in non-reducing SDS sample buffer at a concentration of 10 Ã 10^7 sperm/ml and heated at 60ÂC for 5 min. Lysates were centrifuged to remove insoluble material and supernatants were stored at â80ÂC until used for SDS-PAGE.

Sperm lysates representing 10^6 sperm were reduced by heating for 3 min at 60ÂC in the presence of 40 mM DTT, resolved by SDS-PAGE (Laemmli, 1970), and transferred to nitrocellulose (Towbin et al., 1979). Blots were blocked for 1 h in Tris-buffered saline containing 0.5% Tween-20 and 2.5% non-fat dry milk. CatSper2 polyclonal antibody, directed against the carboxyterminal epitope (Quill et al., 2001) and provided by Dr. Timothy Quill (UT Southwestern) was applied at 1:3000 dilution overnight at 4ÂC. After rinsing, blots were incubated with HRP-conjugated goat anti-rabbit IgG and developed using enhanced chemiluminescence (SuperSignal West Pico, Pierce, Rockford, IL). To assess sample loading, blots were stripped and re-probed using anti-tubulin and a goat anti-mouse IgG-HRP conjugate. Anti-Î-tubulin (mAbE7) developed by Klymkowsky was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biological Sciences, Iowa City, IA 52242.

Sperm Preparation and Experimental Conditions

Sperm were collected as previously described (Suarez and Osman, 1987). Briefly, cauda epididymides were punctured with a 27-gauge needle and incubated at 37ÂC to allow sperm to disperse into surrounding medium. After 10 min, sperm were aspirated and placed in a prewarmed 1.5 ml microcentrifuge tube. Sperm numbers were adjusted to 5 Ã 10^6/ml and sperm were treated with 50 ÎM thimerosal or 5 mM procaine immediately or were incubated for 90 min under capacitating conditions at 37ÂC with 5% CO_2.

Analysis of Sperm Motility

Aliquots of sperm were placed in slide chambers of 50 Îm depth and motility was examined on a 37ÂC stage of a Zeiss Axiovert 35 microscope using differential interference contrast optics with a 40X objective (Carl Zeiss, Inc., Thornbrook, NY) and stroboscopic illumination at 60 Hz provided by a 75 W xenon flash tube (Chadwick-Helmuth Co., El Monte, CA). Videotaping was conducted using a black-and-white Dage CCD 72 video camera (Dage-MTI, Inc., Michigan City, IN) connected to a Panasonic AG-7300 Super VHS videocassette recorder (Panasonic Industrial Co., Secaucus, NY). Videotapes were used to assess the swimming patterns of sperm. For each treatment sample, 100â110 motile sperm were analyzed and, for each experiment, replicate tests were performed using three males from each genotype.

Sperm motility was also evaluated using computer-assisted sperm analysis (CASA). Sperm movement was imaged using a 4X Olympus negative phase objective (Hi-Tech Instruments, Philadelphia, PA) and recorded using a Panasonic AG-7300 Super VHS video recorder. The video images were digitized (30 frames at 60 Hz) and analyzed using HTM-IVOS (Version 10, Hamilton Thorne Biosciences, Beverly, MA). Motion parameters measured were curvilinear velocity (VCL, the rate of travel of the sperm head), amplitude of lateral head displacement (ALH, degree of side-to-side head movement measured as the mean width of head oscillations), and beat/cross frequency (BCF, number of times the head crosses the averaged path in 0.5 sec). Because movement of the sperm head depends on flagellar activity, it can be used as an indicator of flagellar bending patterns. Increased VCL and ALH are

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

indicative of hyperactivation (Mortimer and Mortimer, 1990). For each treatment sample, 400â450 motile sperm were analyzed and for each experiment, replicate tests were performed using three males from each genotype.

Ca^{2+} Image Analysis

Sperm (5 Ã 10^6/ml) were loaded with 5 ÎM of the fluorescent Ca^{2+} indicator dye fluo3 AM (in 0.5% DMSO) for 40 min at 37ÂC in medium lacking bicarbonate and containing 0.6% BSA. Extracellular dye was removed by centrifugation at 170 Ã *g* for 5 min. Sperm were resuspended in medium and incubated for an additional 20 min to allow de-esterification of the dye into its charged, Ca^{2+}-sensitive form. The fluorescence of individual motile sperm in an open micro-chamber at 37ÂC (PDMI-2 Micro-Incubator, Medical Systems Corp. Greenvale, NY) was monitored before and after application of treatments. Sperm were tethered by the head to a glass coverslip in the floor of the chamber by adding them to the chamber in medium lacking BSA, then flooding the chamber with complete medium. Fluorescence intensity was detected with an epifluorescence microscope using a 480 Â 40 nm excitation filter, 535 Â 50 nm emission filter, and a 505 nm long-pass dichroic mirror (Chroma Technology Corp., Rockingham, VT) with an oil immersion 40X Fluar objective (n.a. 1.3, Carl Zeiss Inc.). Stroboscopic illumination was provided by a 75 W xenon arc flash lamp. Images were captured with a Sensicam High Performance camera (The Cooke Corporation, Auburn Hills, MI) and digitized at 2 images/sec controlled by IPLab Spectrum software (Signal Analytics, Vienna, VA).

Data Analysis

Data were analyzed using Minitab statistical software (Minitab Inc., State College, PA). Treatment effects were detected using analysis of variance, followed by Tukeyâs test for individual post-hoc comparisons and were considered statistically significant when $P < 0.05$.

RESULTS

CatSper protein content in sperm from null and heterozygous males

Western blot analysis of sperm samples from heterozygous and homozygous null males with anti-CatSper2 antibody confirmed that sperm from *CatSper*1 and 2 homozygous null males both lacked CatSper2 (Carlson et al., 2005) and that sperm from *CatSper* +/â males contained the same amount of CatSper2 protein as those from *CATSPER* +/+ males (Fig 1).

Release of Ca^{2+} from internal stores is sufficient to initiate hyperactivation in wild-type sperm

Thimerosal (50 ÎM) induced 90â100% of motile wild-type sperm to hyperactivate (Suppl Movie 1B) compared to a 5â10% baseline in controls (Suppl Movie 1A). This effect was detected by CASA as increases in mean ALH, which indicates increased flagellar bend amplitude, and VCL (Table 1). After 5 min exposure to thimerosal, motility declined and sperm arrested with curved flagellum (Fig. 2). Stimulation by thimerosal was not blocked by lowering extracellular Ca^{2+} with 10 mM BAPTA to approximately 30 nM (calculated using MaxChelator: www.stanford.edu/Ëcpatton/maxc.html), which is below the 50 nM intracellular Ca^{2+} level of non-hyperactivated sperm with normal activated (i.e., progressive) motility (Suarez and Dai, 1995;Ho et al., 2002; Table 1).

Fluorescent imaging of fluo3-loaded sperm demonstrated that thimerosal raised Ca^{2+} in the head and flagellum in less than 25 sec (Fig. 3).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Elevation of sperm Ca^{2+}overcomes the motility defect in CatSper1 or CatSper2 null sperm

Analysis of video recorded images of free-swimming sperm revealed abnormal activated motility in both CatSper1 and CatSper2 null sperm. *CATSPER1*â/â sperm swam in circular trajectories instead of normal linear trajectories. The flagella generated curves of long wavelength and moderate amplitude in the direction of the curvature of the head (i.e., the principal bends) and minimal reverse bends (Fig. 4C, Suppl Movies 2A,D). The flagellar beating patterns of *CATSPER2*â/â sperm were similar to those of *CATSPER1*â/â sperm, but the sperm swam in straighter paths, primarily due to periodic rolling along the long axis (Fig. 4D, Suppl Movies 3A,D). These movement patterns were evident immediately after extraction of sperm from epididymides and were maintained throughout a 90 min incubation under capacitating conditions.

Exposure of CatSper null sperm to 50 ÎM thimerosal visibly decreased the asymmetry of the flagellar beat while increasing the bend amplitude (Suppl Movies 2B,E and 3B,E), detected by CASA as increased ALH and VCL (Tables 2 and 3). This produced straighter swimming trajectories in most sperm. The beat frequencies of *CATSPER1*â/â (Table 2) and *CATSPER2*â/â (Table 3) sperm were decreased, causing flagellar activity to resemble that of normal, activated *CATSPER*+/â sperm. In addition, 20â40% of the *CATSPER1*â/â and *CATSPER2*â/â sperm hyperactivated in response to thimerosal, albeit to a lesser degree than *CATSPER*+/â sperm, of which 90â100% hyperactivated. These responses of the null sperm were detected by CASA as increased mean ALH and VCL (Tables 2,3). Addition of 1 mM Ni^{2+} reduced the proportion of both *CATSPER2*+/â and *CATSPER2*â/â sperm exhibiting hyperactivated motility (Fig. 5).

Procaine (5 mM) stimulated hyperactivation in 90% of *CATSPER*+/â sperm but did not hyperactivate *CATSPER1*â/â (Table 2, Suppl Movies 2C,F) or *CATSPER2*â/â (Table 3, Suppl Movies 3C,F) sperm.

Reduction of intracellular Ca^{2+} increases beat frequency and decreases flagellar bend amplitude

Incubation of wild-type sperm for 90 min under capacitating conditions led to the development of hyperactivation, detected by CASA as increased mean ALH and VCL (Table 4). When Ca was omitted from the medium, sperm had lower VCL and ALH as well as higher BCF, and only 17.0 Â 1.2% of sperm hyperactivated compared to 63.3 Â 3.2% in the presence of 2.4 mM added Ca.

To further reduce intracellular Ca^{2+}, wild-type sperm were exposed to a membrane permeable Ca^{2+} chelator, BAPTA AM. This treatment further dampened the flagellar bend amplitude (ALH) and caused a greater increase in BCF (Table 5) while maintaining progressive sperm motility.

DISCUSSION

All sperm from *CATSPER*+/â mice behaved as though they had inherited the wild-type *CATSPER* gene, because gene products are shared through cytoplasmic bridges in developing germ cells (Braun et al., 1989; Ventala et al., 2003). Nearly all sperm from the heterozygotes hyperactivated in response to procaine, rather than half, as would be expected if the *CATSPER* gene products were not shared. The levels of CatSper2 protein, and presumably CatSper1 protein, were the same in sperm from heterozygotes as from wild-type males (Fig 1). It had previously been demonstrated that disruption of either *CATSPER1* or *CATSPER2* genes resulted in the absence of both CatSper1 and CatSper2 proteins in the sperm flagellum (Carlson et al., 2005).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

The results presented here underscore the importance of extracellular Ca^{2+} in the regulation of both activated and hyperactivated motility. Release of Ca^{2+} from internal stores induced the transition from activated to hyperactivated motility in *CATSPER*$^{+/â}$ and *CATSPER*$^{â/â}$ sperm, but extracellular Ca^{2+} was required to sustain hyperactivation. Moreover, lack of CatSper 1 and 2 resulted in flagellar activity that resembled the flagellar movements of wild-type sperm treated to lower intracellular Ca^{2+}; however, motility resembling that of normal activated wild-type sperm could be elicited in the null sperm when internal Ca^{2+} was elevated by release of stores.

Release of internal store Ca^{2+} was sufficient to initiate hyperactivation in wild-type sperm. Similar to the results from bull sperm (Ho and Suarez, 2001), thimerosal quickly induced hyperactivation in the majority of wild-type mouse sperm, even in the absence of available extracellular Ca^{2+}. Thimerosal is known to stimulate release of Ca^{2+} from internal stores (Miyazaki et al., 1992). Ca^{2+} stores gated by IP_3R have been identified at the acrosome (Walensky and Snyder, 1995;Herrick et al., 2005) and base of the flagellum (Ho and Suarez, 2001;Ho and Suarez, 2003), which correspond to the sites of increased Ca^{2+} seen in response to thimerosal in our experiments. Additionally, hyperactivation was elicited by thimerosal in mouse sperm lacking CatSper1 and 2, further implicating internal stores and not an external Ca^{2+} entry pathway in providing Ca^{2+} to initiate hyperactivation.

Interestingly, procaine did not stimulate hyperactivation in *CATSPER1*$^{â/â}$ or *CATSPER2*$^{â/â}$ sperm, consistent with the dependence of procaine-induced hyperactivation on extracellular Ca^{2+} (Marquez and Suarez, 2004). This suggests that procaine activates Ca^{2+} channels at the plasma membrane. Previous analysis of bending in the midpiece indicated that procaine increases flagellar beat asymmetry of tethered *CATSPER2*$^{â/â}$ sperm held at room temperature, although not in a manner identical to hyperactivation (Carlson et al., 2005). Our CASA data and observations of movement patterns of free-swimming sperm at 37ÂC (mouse body temperature) did not reveal hyperactivation in *CATSPER2*â/â sperm in response to procaine. Nonetheless, the ability of thimerosal to induce hyperactivation in the null sperm agrees with the assertion that these sperm are capable of responding to intracellular Ca^{2+} signals and modulating flagellar activity accordingly.

It is evident that extracellular Ca^{2+} is required to maximize and sustain hyperactivation because, without added Ca in the medium, wild-type sperm showed only minimal development of hyperactivation over time. Additionally, 1 mM Ni^{2+} reduced hyperactivation elicited by thimerosal in *CATSPER2*$^{+/â}$ and *CATSPER2*$^{â/â}$ sperm. High Ni^{2+} doses have been shown to block store-operated channels (SOC) in sperm (OâToole et al., 2000;Yoshida et al., 2003). Emptying of store Ca^{2+} is thought to trigger Ca^{2+} influx to refill the store, presumably through SOC at the plasma membrane (Putney, 1986;Berridge 1995;Berridge et al., 2000). TRPC channels are candidates for SOC (Clapham, 2003). It has already been demonstrated that TRPC2, which is localized to the sperm head, provides sustained Ca^{2+} entry during the zona pellucida-induced acrosome reaction (Jungnickel et al., 2001). Furthermore, TRPC1 and TRPC3 have been localized to the midpiece and principal piece, respectively; of mouse sperm (Trevino et al., 2001) and therefore depletion of store Ca^{2+} could activate local SOC in the flagellum to promote hyperactivation. There is also evidence indicating that chemotactic behavior, which consists of asymmetrical flagellar movements in ascidian sperm, is induced by a SOC-mediated increase in intracellular Ca^{2+} (Yoshida et al., 2003).

Elevation of intracellular Ca^{2+} overcame the motility defect in CatSper1 and 2 null sperm. Sperm from *CATSPER1*$^{â/â}$ and *CATSPER2*$^{â/â}$ males expressed abnormal activated motility consisting of low flagellar bend amplitude and higher beat frequency, as previously reported (Carlson et al., 2003;Carlson et al., 2005). We show here for the first time that the abnormal

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

activity might be explained by abnormally low cytoplasmic Ca^{2+}. The abnormal motility could be corrected by thimerosol, presumably by releasing store Ca^{2+} to raise cytoplasmic levels.

Ca^{2+} acts through calmodulin on the axoneme to modulate flagellar curvature (Ho et al., 2002) and blocking the effects of Ca^{2+} with calmodulin inhibitor W13 produces a similar pattern as seen in the CatSper null sperm (Aoki et al., 1999;Tash and Mean, 1982). Moreover, detergent-demembranated sperm display curved flagella with low amplitude bending and higher beat frequency when reactivated under Ca^{2+}-deficient conditions (Lindemann and Goltz, 1988;Lindemann et al., 1987). Hence, *CATSPER1*$^{â/â}$ or *CATSPER2*$^{â/â}$ sperm are likely to contain lower basal cytosolic Ca^{2+} in the flagellum such that thimerosal raised cytosolic Ca^{2+} to the level required to support normal activated motility. In some thimerosol-treated sperm, the rise in intracellular Ca^{2+} apparently overshot the amount needed to produce normal progressive, activated motility and caused them to hyperactivate.

Intracellular Ca^{2+} is approximately 30â50 nM in sperm exhibiting activated motility but 200â1000 nM in hyperactivated sperm (Suarez and Dai, 1995;Ho et al., 2002). Therefore, the decreased effectiveness of thimerosol in sperm lacking CatSper1 or CatSper2 could be because cytosolic Ca^{2+} is maintained at lower levels than normal, making the extent of Ca^{2+} elevation required to initiate hyperactivation even greater.

Whereas both *CATSPER1*$^{â/â}$ and *CATSPER2*$^{â/â}$ sperm were observed to express a similar mildly asymmetrical flagellar beating, *CATSPER1*$^{â/â}$ sperm swam in circles on the slides while *CATSPER1*$^{â/â}$ swam in general straight trajectories due to rolling on the longitudinal axis. The greater tendency of CatSper1 null sperm to be captured by the surface of the glass slide is likely due to its more subdued movement, exemplified as apparently lower VCL, ALH, and BCS (Tables 2,3). Capture of sperm by flat surfaces suppresses torsion in the neck and confines flagellar waves to a single plane; that is, it suppresses rolling (Woolley, 2003). Because *CATSPER1* null sperm lack CatSper2 protein and *vice versa* (Carlson et al., 2005), the differences in the vigor of movement seen in sperm from *CATSPER1* and *CATSPER2* nulls and heterozygotes are attributable to differences in genetic background. In the second report on the phenotype of CatSper1 null sperm (Carlson et al., 2003), the authors noted that the sperm were more vigorous than those described in first report (Ren et al., 2001) and they attributed the difference to changes in genetic background due to backcrossing with mice from another strain.

Ca^{2+} entry through a CatSper2-dependent pathway has been proposed to influence basal cAMP levels because higher basal cAMP is associated with accelerated flagellar beat frequency of *CATSPER2*$^{â/â}$ sperm (Carlson et al., 2005). It is unclear how cAMP generates such a flagellar response when experiments using detergent permeabilized sperm indicate that flagellar curvature and beat frequency of motile sperm are not dependent on cAMP concentrations (Ho et al., 2002). Nevertheless, cAMP signaling of phosphorylation is critical for initiation of motility (Somanath et al., 2004). It is interesting that, unlike sperm of mutant mice lacking soluble adenylyl cyclase (Hess et al., 2005; Esposito et al., 2002), sperm from CatSper1 and CatSper2 null males were highly motile when extracted from the epididymis. Thus, Ca^{2+} signaling mediated by CatSper1 and 2 may not play a major role in initiation of motility in the presence of cAMP but rather in modulating the amplitude of the principal flagellar bend.

In conclusion, the results of this study suggest that, whereas internal Ca^{2+} stores could provide sufficient Ca^{2+} for the induction of hyperactivation, Ca^{2+} influx is required to maintain intracellular Ca^{2+} levels sufficient to sustain hyperactivation. Also, the absence of CatSper1 and 2 may cause a deficiency of Ca^{2+} influx necessary to maintain levels that promote normal activated and hyperactivated flagellar beat patterns and therefore, the inability of sperm to hyperactivate is not the primary defect in CatSper1 and CatSper2 null mutants.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Drs. Timothy Quill and David Garbers, University of Texas Southwestern Medical Center, for providing *CATSPER* heterozygote and homozygote null mutant male mice, sperm samples, and antibody to CatSper2. This work was supported by National Science Foundation grant 39034 to S.S.S. and NIH predoctoral fellowship to B.M.

References

Aoki F, Sakai S, Kohmoto K. Regulation of flagellar bending by cAMP and Ca^{2+} in hamster sperm. Mol Reprod Dev 1999;53:77â83. [PubMed: 10230819]

Berridge MJ. Capacitative calcium entry. Biochem J 1995;312:1â11. [PubMed: 7492298]

Berridge MJ, Lipp P, Boatman MD. The calcium entry pas de deux. Science 2000;287:1604â1605. [PubMed: 10733429]

Braun RE, Behringer RR, Peschon JJ, Brinster RL, Palmiter RD. Genetically haploid spermatids are phenotypically diploid. Nature 1989;337:373â376. [PubMed: 2911388]

Carlson AE, Quill TA, Westenbroek RE, Schuh SM, Hille B, Babcock DF. Identical phenotypes of Catsper1 and Catsper2 null sperm. J Biol Chem 2005;280:32238â32244. [PubMed: 16036917]

Carlson AE, Westenbroek RE, Quill T, Ren D, Clapham DE, Hille B, Garbers DL, Babcock DF. CatSper1 required for evoked Ca^{2+} entry and control of flagellar function in sperm. Proc Natl Acad Sci USA 2003;100:14864â14868. [PubMed: 14657352]

Castellano LE, Trevino CL, Rodriquez D, Serrano CJ, Pacheco J, Tsutsumi V, Felix R, Darszon A. Transient receptor potential (TRPC) channels in human sperm: expression, cellular localization and involvement in the regulation of flagellar motility. FEBS Lett 2002;541:69â74. [PubMed: 12706821]

Clapham DE. TRP channels as cellular sensors. Nature 2003;426:517â524. [PubMed: 14654832]

Esposito G, Jaiswal BS, Xie F, Krajnc-Franken MAM, Robben TJAA, Strik AM, Kuil C, Philipsen RLA, Van Duin M, Conti M, Gossen JA. Mice deficient for soluble adenylyl cyclase are infertile because of a severe sperm-motility defect. Proc Natl Acad Sci USA 2004;101:2993â2998. [PubMed: 14976244]

Fraser LR. Motility patterns in mouse spermatozoa before and after capacitation. J Exp Zool 1977;202:439â444. [PubMed: 591896]

Handrow RR, First NL, Parrish JJ. Calcium requirement and increased association with bovine sperm during capacitation by heparin. J Exp Zool 1989;25:174â182. [PubMed: 2600560]

Herrick SB, Schweissinger DL, Kim SW, Bayan KR, Mann S, Cardullo RA. The acrosomal vesicle of mouse sperm is a calcium store. J Cell Physiol 2005;202:663â671. [PubMed: 15389568]

Hess KC, Jones BH, Marquez B, Chen Y, Ord TS, Kamenetsky M, Miyamoto C, Kopf GS, Suarez SS, Levin LR, Williams CJ, Buck J, Moss SB. Bicarbonate responsive âsolubleâ adenylyl cyclase is responsible for multiple specific signaling events in mammalian sperm required for fertilization. Dev Cell 2005;9:249â259. [PubMed: 16054031]

Ho HC, Granish KA, Suarez SS. Hyperactivated motility of bull sperm is triggered at the axoneme by Ca^{2+} and not cAMP. Dev Biol 2002;250:208â217. [PubMed: 12297107]

Ho HC, Suarez SS. An inositol 1,4,5-trisphosphate receptor-gated intracellular Ca^{2+} store is involved in regulating sperm hyperactivated motility. Biol Reprod 2001;65:1606â1615. [PubMed: 11673282]

Ho HC, Suarez SS. Characterization of the intracellular calcium store at the base of the sperm flagellum that regulates hyperactivated motility. Biol Reprod 2003;68:1590â1596. [PubMed: 12606347]

Jungnickel MK, Marrero H, Birnbaumer L, Lemos JR, Florman HM. Trp2 regulates entry of Ca^{2+} into mouse sperm triggered by egg ZP3. Nat Cell Biol 2001;3:499â502. [PubMed: 11331878]

Kirichok Y, Navarro B, Clapham DE. Whole-cell patch-clamp measurements of spermatozoa reveal an alkaline-activated Ca2+ channel. Nature 2006;439:737â740. [PubMed: 16467839]

Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680â685. [PubMed: 5432063]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Lindemann CB, Goltz JS. Calcium regulation of flagellar curvature and swimming pattern in Triton X-100-extracted rat sperm. Cell Motil Cytoskeleton 1988;10:420â431. [PubMed: 3180254]

Lindemann CB, Goltz JS, Kanous KS. Regulation of activation state and flagellar wave form in epididymal rat sperm: evidence for the involvement of both Ca^{2+} and cAMP. Cell Motil Cytoskeleton 1987;8:324â332. [PubMed: 2826020]

Marquez B, Suarez SS. Different signaling pathways in bovine sperm regulate capacitation and hyperactivation. Biol Reprod 2004;70:1626â1633. [PubMed: 14766720]

Miyazaki S, Shirakawa H, Nakada K, Honda Y, Yuzaki M, Nakade S, Mikoshiba K. Antibody to the inositol trisphosphate receptor blocks thimerosal enhanced Ca^{2+}-induced Ca^{2+} release and Ca^{2+} oscillations in hamster eggs. FEBS Lett 1992;309:180â184. [PubMed: 1324195]

Mortimer ST, Mortimer D. Kinetics of human spermatozoa incubated under capacitating conditions. J Androl 1990;11:195â203. [PubMed: 2384341]

OâToole CMB, Arnoult C, Darszon A, Steinhardt RA, Florman HM. Ca^{2+} entry through store-operated channels in mouse sperm is initiated by egg ZP3 and drives the acrosome reaction. Mol Biol Cell 2000;11:1571â1584. [PubMed: 10793136]

Putney JW. A model for receptor-regulated calcium entry. Cell Calcium 1986;7:1â12. [PubMed: 2420465]

Quill TA, Ren D, Clapham DE, Garbers DL. A voltage-gated ion channel expressed specifically in spermatozoa. Proc Natl Acad Sci USA 2001;98:12527â12531. [PubMed: 11675491]

Quill TA, Sugden SA, Rossi KL, Doolittle LK, Hammer RE, Garbers DL. Hyperactivated sperm motility driven by CatSper2 is required for fertilization. Proc Natl Acad Sci USA 2003;100:14869â14874. [PubMed: 14657366]

Ren D, Navarro B, Perez G, Jackson AC, Hsu S, Shi Q, Tilly JL, Clapham DE. A sperm ion channel required for sperm motility and male fertility. Nature 2001;413:603â609. [PubMed: 11595941]

Somanath PR, Jack SL, Vijayaraghavan S. Changes in sperm glycogen synthase kinase-3 serine phosphorylation and activity accompany motility initiation and stimulation. J Androl 2004;25:605â617. [PubMed: 15223849]

Stauss CR, Votta TJ, Suarez SS. Sperm motility hyperactivation facilitates penetration of the hamster zona pellucida. Biol Reprod 1995;52:1280â1285. [PubMed: 8562682]

Suarez SS, Dai X. Intracellular calcium reaches different levels of elevation in hyperactivated and acrosome-reacted hamster sperm. Mol Reprod Dev 1995;42:325â333. [PubMed: 8579847]

Suarez SS, Katz DF, Overstreet JW. Movement characteristics and acrosomal status of rabbit spermatozoa recovered at the site and time of fertilization. Biol Reprod 1983;29:1277â1287. [PubMed: 6652189]

Suarez SS, Osman RA. Initiation of hyperactivated flagellar bending in mouse sperm within the female reproductive tract. Biol Reprod 1987;36:1191â1198. [PubMed: 3620562]

Tash JS, Means AR. Regulation of protein phosphorylation and motility of sperm by cyclic adenosine monophosphate and calcium. Biol Reprod 1982;26:745â763. [PubMed: 6282354]

Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci U S A 1979;76:4350â4354. [PubMed: 388439]

Trevino CL, Felix R, Castellano LE, Gutierrez C, Rodriquez D, Pacheco J, Lopez-Gonzalez I, Gomora JC, Tsutsumi V, Hernandez-Cruz A, Fiordelisio T, Scaling AL, Darszon A. Expression and differential cell distribution of low-threshold Ca^{2+} channels in mammalian male germ cells and sperm. FEBS Lett 2004;563:87â92. [PubMed: 15063728]

Trevino CL, Serrano CJ, Beltran C, Felix R, Darszon A. Identification of mouse trp homologs and lipid rafts from spermatogenetic cells and sperm. FEBS Lett 2001;509:119â125. [PubMed: 11734218]

Ventela S, Toppari J, Parvinen M. Intercellular organelle traffic through cytoplasmic bridges in early spermatids of the rat: mechanisms of haploid gene product sharing. Mol Biol Cell 2003;14:2768â2780. [PubMed: 12857863]

Walensky LD, Snyder SH. Inosital 1,4,5-trisphosphate receptors selectively localized to the acrosomes of mammalian sperm. J Cell Biol 1995;130:857â869. [PubMed: 7642703]

Wennemuth G, Westenbroek RE, Xu T, Hille B, Babcock DF. $Ca_V2.2$ and $Ca_V2.3$ (N- and R-type) Ca^{2+} channels in depolarization-evoked entry of Ca^{2+} into mouse sperm. J Biol Chem 2000;275:21210â21217. [PubMed: 10791962]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Wiesner B, Weiner J, Middendorff R, Hagen V, Kaupp UB, Weyand I. Cyclic nucleotide-gated channels on the flagellum control Ca^{2+} entry into sperm. J Cell Biol 1998;142:473â484. [PubMed: 9679145]

Woolley DM. Motility of spermatozoa at surfaces. Reproduction 2003;126:259â270. [PubMed: 12887282]

Yanagimachi, R. Mammalian fertilization. In: Knobil, E.; Neill, J., editors. The Physiology of Reproduction. Raven Press; New York: 1994. p. 189-317.

Yanagimachi R. The movement of golden hamster spermatozoa before and after capacitation. J Reprod Fert 1970;23:193â196.

Yoshida M, Ishikawa M, Izumi H, De Santis R, Morisawa M. Store-operated calcium channel regulates the chemotactic behaviour of ascidian sperm. Proc Natl Acad Sci USA 2003;100:149â154. [PubMed: 12518063]

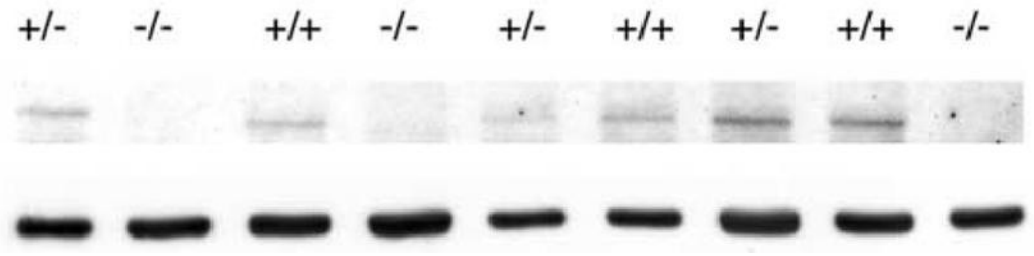

FIG 1.

Immunoblots demonstrating that sperm from *CATSPER2*+/â males contain the same levels of CatSper2 protein as sperm from wild-type (*CATSPER2*+/+) males. Upper blot, sperm extracts labeled with CatSper2 antibody. Lower blot, after removing CatSper2 antibody, blots were probed with anti-tubulin antibody to indicate numbers of sperm loaded per lane.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

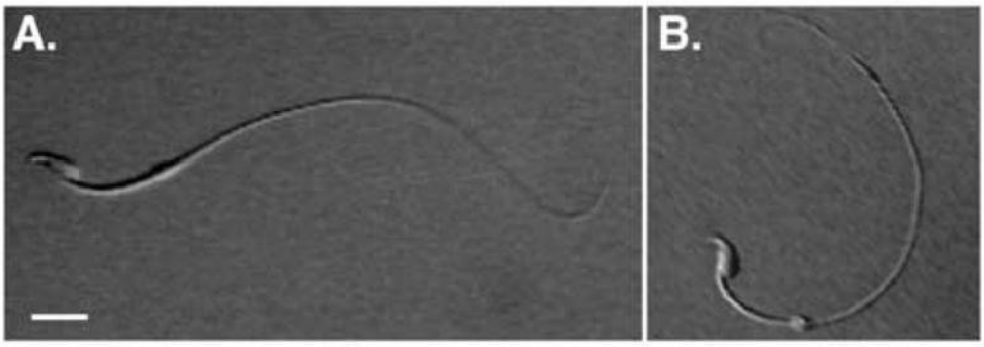

FIG 2.

Flagellar curvature generated by thimerosal. Activated sperm swimming in medium alone (A). Prolonged exposure to thimerosal caused sperm to arrest with the flagellum curved in the direction opposite of the curve of the head (B). Scale bar = 10 Îm.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

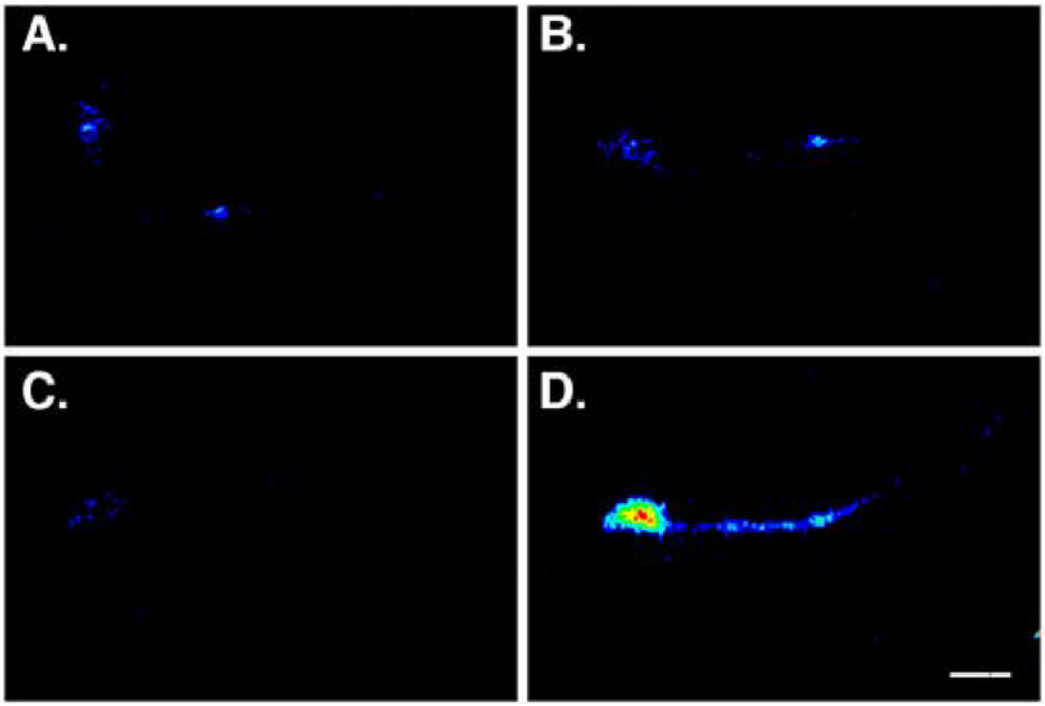

FIG 3.

Ca^{2+} imaging of fluo3-loaded sperm. Images of wild-type sperm were captured before (A, C) and 25 sec after (B, D) application of medium alone (B) or containing thimerosal (D). Warmer colors indicate higher fluorescent intensities and increased intracellular Ca^{2+}. Scale bar = 10 Îm.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

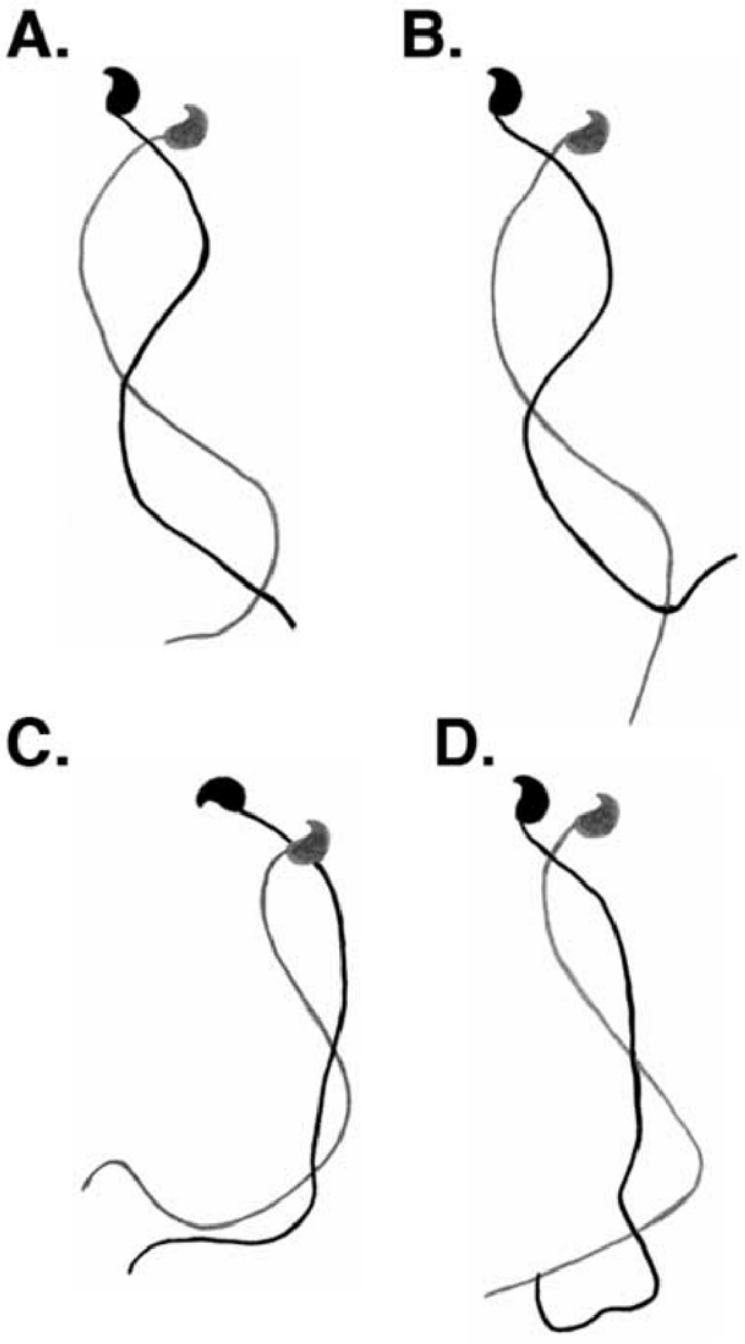

FIG 4.

Flagellar beating patterns of *CATSPER1*$^{â/â}$ and *CATSPER2*$^{â/â}$ sperm. *CATSPER1*$^{+/â}$ (A) and *CATSPER2*$^{+/â}$ (B) sperm display normal activated motility consisting of moderate amplitude flagellar bends and nearly symmetrical beating. *CATSPER1*$^{â/â}$ (C) and *CATSPER2*$^{â/â}$ (D) sperm display low bending amplitude and slightly asymmetrical flagellar beating (D). Black and gray indicate the principal and the reverse flagellar bends, respectively.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

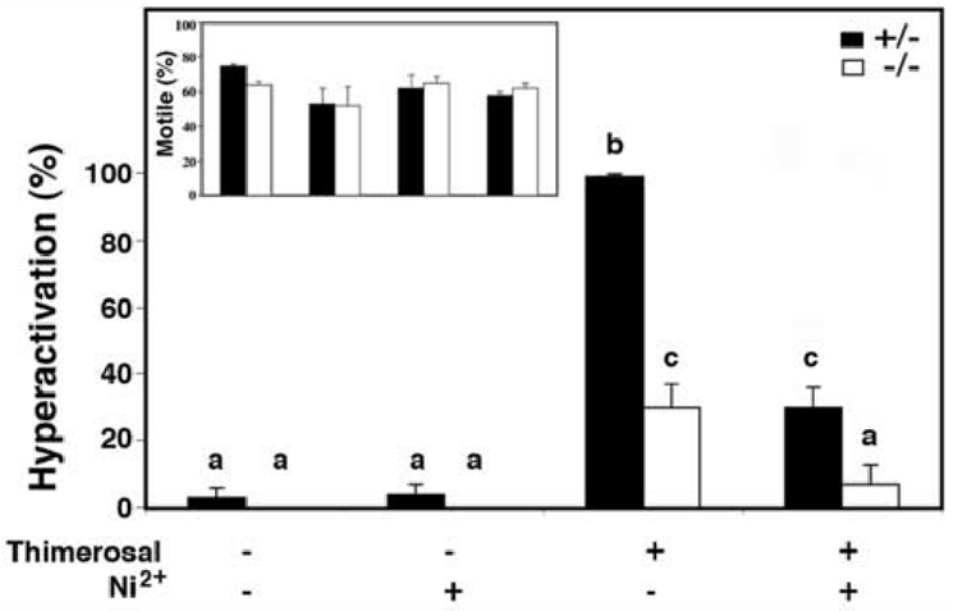

FIG 5.

Ni^{2+} reduced thimerosal-induced hyperactivation. *CATSPER2*$^{+/â}$ or *CATSPER2*$^{â/â}$sperm were treated with thimerosal after 5 min pre-incubation in 1 mM Ni^{2+}. Bars labeled with different letters indicate significant differences between treatments ($P < 0.05$).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

TABLE 1

CASA measurements of effects of Ca^{2+} and thimerosal on sperm motility.[a]

	MotileÂ(%)	VCLÂ(Îm/sec)	ALHÂ(Îm)	BCFÂ(/sec)
Control	83.0ÂÂÂ2.1	331.8ÂÂÂ10.2	14.8ÂÂÂ0.6	27.5ÂÂÂ0.5
â+ÂBAPTA	83.3ÂÂÂ1.9	265.6ÂÂÂ3.6[b]	11.6ÂÂÂ0.4[b]	31.8ÂÂÂ0.4[b]
Thimerosal	75.7ÂÂÂ6.3	392.0ÂÂÂ15.0[c]	23.2ÂÂÂ0.2[c]	23.6ÂÂÂ2.5
â+ÂBAPTA	78.0ÂÂÂ1.2	391.9ÂÂÂ4.1[c]	24.4ÂÂÂ0.5[c]	20.8ÂÂÂ1.4[c]

[a] Sperm were incubated in medium containing 2.4 mM Ca or 1.2 mM Ca + 10 mM BAPTA in the absence or presence of 50 ÎM thimerosal for 1 min. (mean Â SEM).

[b] Different from all other treatments ($P < 0.05$).

[c] Different from control ($P < 0.05$).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

TABLE 2

CASA measurements of *CATSPER1* sperm treated with thimerosal or procaine.[a]

	MotileÂ(%)		VCLÂ(Îm/sec)		ALHÂ(Îm)		BCFÂ(/sec)	
	+/â	â/â	+/â	â/â	+/â	â/â	+/â	
Control	69ÂÂÂ1	68ÂÂÂ3	306.7ÂÂÂ12.0	214.6ÂÂÂ0.5[d]	14.7ÂÂÂ0.9	9.6ÂÂÂ0.3[d]	30.0ÂÂÂ0.3	36.4Â
Thimerosal	69ÂÂÂ3	64ÂÂÂ2	346.9ÂÂÂ10.0[b]	289.7ÂÂÂ5.3,[bd]	20.1ÂÂÂ0.6[b]	18.4ÂÂÂ0.2,[bd]	22.4ÂÂÂ2.0[b]	25.0Â
Procaine	62ÂÂÂ2	61ÂÂÂ2	381.7ÂÂÂ2.1,[bc]	216.6ÂÂÂ4.8,[cd]	18.6ÂÂÂ0.5,[bc]	9.0ÂÂÂ0.1,[cd]	33.9ÂÂÂ1.7,[bc]	39.7Â

[a] Sperm were incubated in medium alone or containing 50 ÎM thimerosal or 5 mM procaine for 1 min (mean Â SEM).

[b] Different from control ($P < 0.05$).

[c] Different from thimerosal ($P < 0.05$).

[d] Different from +/â ($P < 0.05$).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

TABLE 3

CASA measurements of *CATSPER2* sperm treated with thimerosal or procaine.[a]

	MotileÂ(%)		VCLÂ(Îm/sec)		ALHÂ(Îm)		BCFÂ(/sec)	
	+/â	â/â	+/â	â/â	+/â	â/â	+/â	
Control	75ÂÂÂ1	63ÂÂÂ2	348.5ÂÂÂ5.0	265.1ÂÂÂ9.1[d]	16.8ÂÂÂ0.4	12.0ÂÂÂ0.5[d]	29.3ÂÂÂ0.2	40.0Â
Thimerosal	62ÂÂÂ3	68ÂÂÂ1	415.4ÂÂÂ3.4[b]	359.1ÂÂÂ9.1,[bd]	23.5ÂÂÂ1.0[b]	18.1ÂÂÂ0.5,[bd]	20.8ÂÂÂ1.9[b]	25.5Â
Procaine	60ÂÂÂ1	64ÂÂÂ1	432.2ÂÂÂ6.1,[bc]	307.0ÂÂÂ7.7,[bcd]	22.2ÂÂÂ0.4[b]	12.5ÂÂÂ1.0,[cd]	30.9ÂÂÂ0.7,[bc]	44.1Â

[a] Sperm were incubated in medium alone or containing 50 ÎM thimerosal or 5 mM procaine for 1 min (mean Â SEM).

[b] Different from control ($P < 0.05$).

[c] Different from thimerosal ($P < 0.05$).

[d] Different from +/â ($P < 0.05$).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

TABLE 4

CASA measurements of effects of extracellular Ca^{2+} on capacitation-induced hyperactivation.[a]

CaÂ(mM)	MotileÂ(%)	VCLÂ(Îm/sec)	ALHÂ(Îm)	BCFÂ(/sec)
2.4	59.7ÂÂÂ0.9	366.8ÂÂÂ8.1	18.5ÂÂÂ0.3	21.7ÂÂÂ1.0
0.0	71.3ÂÂÂ1.2[b]	256.3ÂÂÂ4.5[b]	13.8ÂÂÂ0.5[b]	27.9ÂÂÂ1.7[b]

[a] Sperm were incubated in medium with or without 2.4 mM added Ca for 90 min (mean Â SEM).

[b] Different from 2.4 mM Ca ($P < 0.05$).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

TABLE 5

CASA measurements of effects of reduced intracellular Ca^{2+} on sperm motility.[a]

	MotileÂ(%)	VCLÂ(Îm/sec)	ALHÂ(Îm)	BCFÂ(/sec)
Control	58.3ÂÂÂ1.9	346.9ÂÂÂ6.6	16.7ÂÂÂ0.5	27.3ÂÂÂ1.1
BAPTAÂAM	65.3ÂÂÂ3.5	198.7ÂÂÂ4.0[b]	8.7ÂÂÂ0.4[b]	37.7ÂÂÂ0.6[b]

[a] Sperm were incubated in medium containing 0.25% DMSO alone or 25 ÎM BAPTA AM in 0.25% DMSO for 15 min (mean Â SEM).

[b] Different from control ($P < 0.05$).