Published in final edited form as:
Mol Reprod Dev. 2012 January ; 79(1): 4–18. doi:10.1002/mrd.21393.

Heads or Tails? Structural Events and Molecular Mechanisms That Promote Mammalian Sperm Acrosomal Exocytosis and Motility

Mariano G. Buffone[1], **Takashi W. Ijiri**, **Wenlei Cao**, **Tanya Merdiushev**, **Haig K. Aghajanian**, and **George L. Gerton**
Center for Research on Reproduction and Women's Health, Department of Obstetrics and Gynecology, University of Pennsylvania School of Medicine, Philadelphia, PA USA

Abstract

Sperm structure has evolved to be very compact and compartmentalized to enable the motor (the flagellum) to transport the nuclear cargo (the head) to the egg. Furthermore, sperm do not exhibit progressive motility and are not capable of undergoing acrosomal exocytosis immediately following their release into the lumen of the seminiferous tubules, the site of spermatogenesis in the testis. These cells require maturation in the epididymis and female reproductive tract before they become competent for fertilization. Here we review aspects of the structural and molecular mechanisms that promote forward motility, hyperactivated motility, and acrosomal exocytosis. As a result, we favor a model articulated by others that the flagellum senses external signals and communicates with the head by second messengers to affect sperm functions such as acrosomal exocytosis. We hope this conceptual framework will serve to stimulate thinking and experimental investigations concerning the various steps of activating a sperm from a quiescent state to a gamete that is fully competent and committed to fertilization. The three themes of compartmentalization, competence, and commitment are key to an understanding of the molecular mechanisms of sperm activation. Comprehending these processes will have a considerable impact on the management of fertility problems, the development of contraceptive methods, and, potentially, elucidation of analogous processes in other cell systems.

Keywords

sperm activation; epididymal maturation; hyperactivation; acrosomal exocytosis

Introduction

In the following pages, we consider recent findings in sperm physiology that we feel should be incorporated into paradigms concerning the structure/function features that govern how the highly specialized, streamlined spermatozoon accomplishes its single purpose: to deliver a haploid copy of the male's genome to the egg and initiate embryonic development. For the purposes of our discussion, we emphasize the concepts of compartmentalization, capacitation, and commitment that underlie the competence of a spermatozoon for

[*]**Correspondence and reprint requests:** George L. Gerton, Ph.D. Center for Research on Reproduction and Women's Health Department of Obstetrics and Gynecology University of Pennsylvania Medical Center 421 Curie Blvd., 1311 BRB II/III Philadelphia, PA 19104-6080 gerton@mail.med.upenn.edu Office: (215) 573-4781 Laboratory: (215) 573-4786 Fax: (215) 573-7627.
[1]Current address: Instituto de Biologia y Medicina Experimental, Vuelta de Obligado 2490, Buenos Aires 1428, Argentina.

Quote: "[T]he flagellum plays a unifying role in the structural compartmentalization, functional competence, and commitment to activation in sperm."

fertilization. Key functions, such as acrosomal exocytosis and sperm motility, are structurally compartmentalized within the head and tail of the sperm, respectively. Thus, an explanation of mechanisms that promote these functions must account for their respective localizations, the energetics of these processes, and the signaling pathways regulating them. Furthermore, sperm are unable to perform these functions immediately following their release into the lumen of the seminiferous tubules of the testis. Maturation in specific places and at particular times ultimately enables sperm to become competent for fertilization. In particular, sperm must undergo a process known as capacitation that leads to hyperactivated motility and prepares the sperm for acrosomal exocytosis (Visconti et al., 2011). Capacitation normally takes place with the female reproductive tract, but it can be mimicked in the laboratory by the incubation of sperm in a defined medium. Incubation of mouse sperm in medium containing bicarbonate, a cholesterol-acceptor, calcium, and an energy source can lead to capacitation; sperm from other species may have other requirements. Actual commitment to the irreversible and final phases of fertilization cannot take place unless the fertilizing sperm has undergone the requisite steps to become competent.

In this short review, we bring together recent findings in the field of sperm physiology and synthesize these into a conceptual framework that we hope will serve to stimulate thinking and experimental investigations concerning the various steps of activating a quiescent sperm into a fertilization dynamo. Some of these concepts derive from advanced studies in genomics and proteomics that, over the past decade, have played a pivotal role in elucidating the molecular mechanisms underlying the activation of sperm cells, ciliary and flagellar function in somatic cells and single-celled organisms, and stimulus-secretion coupling in endocrine cells. Specifically, we propose that the sperm flagellum has a role in sensing and signaling to control motility characteristics and to communicate to the head to influence acrosomal exocytosis.

Spermatozoa can be basically divided into two major morphologically distinct compartments critical for fertilization: head and tail (Figure 1) [for a further review, please see Eddy (2006)]. The head is further partitioned into an acrosomal region (encompassing the secretory acrosomal vesicle with its contents and the plasma membrane overlying the acrosome, which are involved in zona pellucida (ZP) binding and acrosomal exocytosis) and a nucleus, which contains a haploid version of the male's genetic material. The flagellum, which produces energy and propels the sperm through the female reproductive tract, is structurally segmented into the mitochondrial midpiece, the principal piece, and the end piece.

A fascinating characteristic of these highly specialized cells is that the transformation to a functionally competent form is achieved during the post-spermatogenic period, when the male germ cell is no longer engaged in gene transcription and mRNA translation. Sperm emerge from the testes in a functionally inactive form and then pass through the epididymis where events, including post-translational modifications (protein sulfhydryl oxidation, modification of carbohydrate moieties of sperm glycoproteins, etc.) and the addition of secreted proteins from the epididymal epithelium, enable the spermatozoa to reach the first stage of cell competence, acquisition of the ability to become progressively motile when the sperm find themselves in a permissive environment, such as the female reproductive tract or an appropriate experimental medium [reviewed by Cornwall (2009)]. Following release into and residence within the female reproductive tract for a period of time, sperm undergo capacitation, which includes biochemical correlates such as the stimulation of soluble adenylyl cyclase activity, protein tyrosine phosphorylation, and protein nitrosylation (Esposito et al., 2004; Hess et al., 2005; Visconti et al., 2011; Machado-Oliveira et al., 2008). Capacitation encompasses the second and third stages of activation: initiation of hyperactivated motility and the acquisition of competence for acrosomal exocytosis in

response to an appropriate physiological stimulus (Suarez, 2008; Mayorga et al., 2007; Zanetti and Mayorga, 2009; Tsai et al., 2010). Sperm penetration of the ZP and the later events of fertilization require acrosomal exocytosis. Successful fertilization relies on the activation of all three stages of functional competence: forward motility, hyperactivated motility, and acrosomal exocytosis. These events must take place in the appropriate spatio-temporal context for a sperm to complete fertilization.

The flagellum and the acrosome are so structurally distinct and physically incongruent that the tendency is to treat the functional activation of flagellar motility and acrosomal exocytosis as unrelated. Although it is possible (as will be discussed below) to consider that aspects of these events are independently controlled, we favor the concept that, under normal physiological circumstances, the flagellum plays a key role in the control of acrosomal exocytosis as well as motility. At present, there are few papers promoting this model (Wood et al., 2003; Xia et al., 2007), but we encourage the reader to consider the possibility that the mammalian sperm flagellum possesses characteristics of a sensory cilium (Bloodgood, 2010). In this manner, the flagellum could sense alterations in the external milieu (such as pH and ion changes) and potentially detect and respond to chemotactic or chemokinetic factors that could influence the direction and speed of movement. An intracellular “sensing” mechanism could also be used to relay an environment-based second messenger signal such as calcium from the flagellum to the head, thereby stimulating acrosomal exocytosis. To build on this concept, we address the three stages of functional competence introduced above (forward motility, hyperactivated motility, and acrosomal exocytosis), and then bring these events together in a model that considers our proposal that the flagellum plays a unifying role in the structural compartmentalization, functional competence, and commitment to activation in sperm.

Acquisition of competence during epididymal maturation: Facilitating motility

After being produced by spermatogenesis in the seminiferous tubules of the testis, sperm pass through the rete testis, the efferent ducts, the caput epididymis, corpus epididymis, and, finally, to the cauda epididymis where they are stored until ejaculation (Cornwall, 2009). During epididymal transit, sperm undergo intrinsic biochemical and physiological modifications that result in the acquisition of progressive motility and the ability to undergo capacitation required for fertilization (Sullivan et al., 2007). On a biochemical level, proteins from caput epididymal sperm contain a greater number of free sulfhydryl groups than disulfide bonds, and the oxidation of sulfhydryl groups during epididymal transit is correlated with the stabilization of sperm tail structures and promotion of tyrosine phosphorylation of sperm proteins involved in the signaling pathways (Cornwall et al., 1988; Calvin and Bedford, 1971; Seligman et al., 2004). Recent work indicates that caput epididymal sperm possess the ability to become motile, but the swimming capability is suppressed via the activity of cannabinoid receptor CNR1; when bound to its ligand, the endocannabinoid 2-arachidonoylglycerol, CNR1 suppresses the swimming ability of sperm from the caput epididymis where 2-arachidonoylglycerol levels are high (Cobellis et al., 2010). Using proteomics, several rat sperm proteins have been identified as candidates that undergo post-translational modifications in the epididymis (Baker et al., 2005). At this point, the mechanism underlying the acquisition of forward motility by cauda epididymal sperm has not been completely elucidated, but the proteins identified in these proteomic analyses provide target molecules for studying the effects of changes in amounts, disulfide bond status, proteolysis, and alterations such as phosphorylation on flagellar function (e.g., wave amplitude, periodicity, form, and response to external signals).

The external environment experienced by sperm changes as they progress through the epididymis. Acidification of the luminal contents of the epididymis maintains sperm in an immotile state and is regulated by epididymal clear cells, which sense a rise in luminal pH or bicarbonate concentrations through the SACY-dependent rise in cAMP, which leads to a lowering of the luminal pH by causing the apical membrane accumulation of the proton secreting V-ATPase (Pastor-Soler et al., 2003; Shum et al., 2009). The sperm encounter an assortment of different secretory proteins as they pass through the various regions of the epididymis, some of which have been proposed to contribute to sperm maturation (Syntin et al., 1996; Dacheux et al., 2003, 2006). Curiously, genetic ablation of one of the most actively studied epididymal secretory proteins, CRISP1, results in no difference in fertility of *Crisp1*$^{-/-}$ male or female mice compared to controls; sperm motility and the ability to undergo a spontaneous or progesterone-induced acrosome reaction are not affected in vivo (Da Ros et al., 2008). In laboratory tests, however, capacitation-associated protein tyrosine phosphorylation and in vitro fertilization of ZP-intact and ZP-free eggs are significantly reduced suggesting that CRISP1 contributes to the fertilization competence of mouse sperm.

Glycolysis and ATP

Like all cells, sperm require the energy of ATP to power cell motility, acrosomal exocytosis, and membrane functions such as ion pumps and channels. With an absence of glycogen and little capacity to store fuel, sperm must make their own ATP from available substrates. During spermatogenesis, a number of somatic-type glycolytic enzymes are replaced by male germ cell-specific isozymes, many of which are found in spermatozoa in association with the fibrous sheath of the flagellar principal piece (McCarrey and Thomas, 1987; Mori et al., 1993; Sakai et al., 1987; Welch et al., 1992) (Fig. 1). Although spermatocytes and spermatids prefer oxidative phosphorylation for ATP production, glycolysis appears to be the dominant pathway in mouse sperm whereas oxidative phosphorylation is the predominant pathway in bull sperm (Storey, 2008). Studies have suggested that, during epididymal maturation, rabbit sperm switch to glycolysis for ATP production (Storey and Kayne, 1975). Glycolysis clearly plays an essential role as an energy pathway to fuel forward motility in mouse sperm since male mice with genetic deletions of the sperm-specific forms of key glycolytic enzymes (glyceraldehyde 3-phosphate dehydrogenase-S or phosphoglyerate kinase-2) are infertile or have very low fertility (Miki et al., 2004; Danshina et al., 2010). In part, this is due to much lower levels of ATP production (4- to 10-fold lower than wild-type sperm), resulting in poor (sluggish) motility characteristics. The spermatogenic cell-specific type 1 hexokinase of the mouse exhibits cleavage of protein disulfide bonds in cauda epididymal sperm, resulting in increased hexokinase activity associated with the initiation of sperm motility (Nakamura et al., 2008). This indicates that protein structural changes during epididymal maturation have functional consequences, improving a sperm's competence for motility.

Glycogen synthase kinase 3 is involved in the initiation of motility

The acquisition of sperm motility is due, in part, to a decline in glycogen synthase kinase-3 (GSK-3) activity as the sperm traverse the epididymis (Fig. 2). GSK-3 activity in immature and immotile bovine caput epididymal sperm is six times higher than that found in motile cauda epididymal sperm (Vijayaraghavan et al., 1996). When immature bovine caput spermatozoa are treated with the phosphatase inhibitors calyculin A or okadaic acid, they acquire motility without affecting intracellular levels of cyclic AMP (cAMP), pH, and calcium. This in vitro initiation of sperm motility dramatically increases serine phosphorylation and coincident inactivation of GSK-3, which is found in both the head and tail of bovine sperm (Somanath et al., 2004). In porcine sperm, the cAMP analogue 8-Br-cAMP was also able to increase GSK-3 serine phosphorylation as well as increasing sperm velocity (Aparicio et al., 2007).

Cyclic AMP and the role of SACY

The major inhibitory effect of serine phosphorylation on the enzymatic activity of GSK-3 is coupled with the enhancement of this modification by the ubiquitous secondary messenger cAMP (Fig. 2). There several ways that cAMP could operate in sperm such as the activation of cAMP-regulated protein kinase A (PKA) or through exchange proteins activated by cAMP (EPACs). There is a strong body of evidence indicating that PKA facilitates the acquisition of motility by cauda epididymal sperm. PKA subunits are compartmentalized into detergent-resistant structures of the midpiece as well as the principal piece of the sperm tail where, they are bound to the fibrous sheath through A-kinase anchoring proteins (AKAPs), two of which – AKAP3 and AKAP4 – are the major proteins of this structure (Carrera et al., 1994; Fulcher et al., 1995; Vijayaraghavan et al., 1999). In sperm null for the RIIα regulatory subunit of PKA, RIα protein is increased and PKA relocalizes to cytoplasmic droplet where it is extractable by detergent (Burton et al., 1999). The RIIα-deficient mutant mice are fertile and display normal sperm motility, indicating that the highly localized pattern of PKA seen in wild-type sperm is not essential for motility or fertilization. In addition, male mice null for the sperm-specific Cα2 catalytic subunit of PKA produce normal numbers of sperm that swim spontaneously in vitro, indicating that Cα2 is not required to form a flagellum or to enable the sperm to acquire motility in the epididymis (Nolan et al., 2004). Yet, these sperm do not respond to bicarbonate with an increase in flagellar beating or facilitated calcium entry, nor do they exhibit capacitation-associated protein tyrosine phosphorylation. In light of these specific defects, it is not surprising that Cα2-null males are infertile even though they display normal mating behavior.

The level of cAMP in a given cell is balanced by the enzymatic activities of adenylyl cyclases that convert ATP to cAMP and phosphodiesterases, which degrade cAMP to AMP. The two general classes of adenylyl cyclases, transmembrane (ADCY) and soluble (SACY), have different biochemical properties. ADCYs are generally G-protein regulated and activated by forskolin (Kamenetsky et al., 2006) whereas SACY is activated by bicarbonate and calcium (Zippin et al., 2001). Although it is present in somatic tissues, SACY is most abundant and its activity is very prevalent in the mammalian testis, and the vast majority of cAMP in developing germ cells and mature sperm is generated by this cyclase (Xie et al., 2006). In cauda epididymal sperm, this protein localizes mainly in the midpiece of the flagellum (Hess et al., 2005).

Sacy-null male mice produced by homologous recombination show normal spermatogenesis, but are infertile because of a severe sperm motility defect (Esposito et al., 2004; Hess et al., 2005). Forward motility in the *Sacy*-null sperm can be restored by adding a cell permeable cAMP analogue, adenosine 3′,5′-cyclic monophosphate acetoxymethyl ester (cAMP-AM). Another cAMP analogue, N6,2′-O-dibutyryladenosine 3′,5′-cyclic monophosphate (dbcAMP), also rescues motility in the *Sacy*-null sperm, although bicarbonate-induced protein phosphotyrosine patterns, hyperactivated motility, and fertilization competence in vitro cannot be restored (Hess et al., 2005). KH7, a novel specific inhibitor of SACY, disturbs motility, protein tyrosine phosphorylation patterns, and fertilization competence of wild-type sperm, but the addition of dbcAMP rescues all of these features. Distinct from effects on motility, acrosomal exocytosis is normal in *Sacy*-null sperm and KH7-treated wild-type sperm. These results reveal that capacitation-associated events (protein tyrosine phosphorylation, hyperactivated motility, acrosomal exocytosis) are not absolutely linked with each other. In addition, the patterns of protein tyrosine phosphorylation that result when these two cAMP analogues are used appear to be different. Further experiments are required to expand our understanding of the role of cAMP in the acquisition of fertilization competence.

In the mouse, ADCY2, ADCY3, and ADCY8 are expressed in sperm whereas lower levels of ADCY1 and ADCY4 are present (Baxendale and Fraser, 2003). In particular, ADCY3 is important in male germ cells where it is located in the acrosomal cap, neck, and flagellum (Livera et al., 2005; Baxendale and Fraser, 2003). Sperm from *Adcy3*$^{-/-}$ mice have decreased motility and show a reduced ability for in vitro fertilization; however, the fertilizing ability can be recovered using ZP-free eggs (Livera et al., 2005). Multiple studies have also demonstrated the existence of G-proteins in the acrosomal region (Glassner et al., 1991; Merlet et al., 1999). These results, coupled with the lack of SACY in the acrosome indicate that ADCY3 plays a pivotal role in acrosomal exocytosis (Fig. 2).

Activation during capacitation: Acquisition of hyperactivated motility

Calcium and CATSPERs

Immediately after collection from the cauda epididymis or an ejaculate, sperm display forward motility when placed in an appropriate medium. After incubation in capacitating conditions in vitro or in the female reproductive tract in vivo, mouse (but not bull) sperm exhibit hyperactivated motility that is characterized by asymmetrical flagellar beats with increased amplitude of the principal flagellar bend (Suarez et al., 1987; Marquez and Suarez, 2007). Hyperactivity requires the alkalinization of the sperm and is calcium-dependent. Calcium can be mobilized into sperm from the external milieu by plasma membrane channels, and it is also released internally from intracellular stores, such as the redundant nuclear envelope located at the base of sperm flagellum or the acrosome (Ho and Suarez, 2003; Herrick et al., 2005; Costello et al., 2009).

A number of calcium channel proteins have been identified in sperm by immunohistochemisty or by measuring specific channel activities, and are regulated by voltage or other mechanisms (Wissenbach et al., 1998; Arnoult et al., 1996; Ren et al., 2001; Escoffier et al., 2007; Pinto et al., 2009). Members of the CATSPER (cation channel, sperm associated) family of calcium channel proteins are sensitive to intracellular alkalinization and are essential for sperm capacitation (Kirichok et al., 2006; Quill et al., 2001; Qi et al., 2007; Lobley et al., 2003; Ren et al., 2001) (Fig. 2). Male mice nullizygous for each of four individual *Catsper* genes are infertile because they are incapable of hyperactivated motility required for ZP penetration; *Catsper*-null female mice, however, remain fertile (Carlson et al., 2005; Jin et al., 2007; Quill et al., 2001; Qi et al., 2007; Ren et al., 2001). A human *CATSPER1* insertional mutation causing a frame shift leads to male infertility (Avenarius et al., 2009). Consistent with the findings above, *Catsper*-null mouse sperm reach the oviductal reservoir, but lack the ability to perform the deep asymmetrical flagellar bends that are required for the sperm to detach from the oviductal epithelium (Ho et al., 2009).

Potassium

How ion changes come about within sperm is an exciting but sometimes perplexing field of study. Intracellular alkalinization during the course of sperm capacitation not only activates CATSPER channels, but it also dramatically affects membrane potential, producing a rapid hyperpolarization of the cell. The hyperpolarization is mediated primarily by a weak outwardly rectifying K^+ current (I_{KSper}) originating from the principal piece of the sperm flagellum (Navarro et al., 2007). Alkalinization activates the pH_i-sensitive I_{KSper}, creating a negative membrane potential where Ca^{2+} entry via $I_{CATSPER}$ is maximized. A time- and voltage-dependent K^+ channel activated by intracellular alkalinization was detected in mouse sperm by patch clamp analysis, and is likely due to KCNU1 (mSlo3) (Martínez-López et al., 2009) (Fig. 2). Another channel potentially involved in hyperpolarization is the K_{ATP} channel. Using a whole-cell patch clamp with mouse spermatogenic cells, an inwardly rectifying K^+ current (K_{ir}) can be recorded, which is sensitive to inhibition by Ba^{2+}, glucose,

and the sulfonylureas (tolbutamide and glibenclamide). K_{ATP} subunit SUR1, SUR2, $K_{ir}6.1$, and $K_{ir}6.2$ transcripts are present in total RNA from elongating spermatids, and immunohistochemistry shows the protein subunits are present in sperm. Of note, incubation of sperm with tolbutamide during capacitation abolishes hyperpolarization and significantly decreases the percentage of acrosomal exocytosis in a dose-dependent fashion (Acevedo et al., 2006). In contrast, a previous report indicated that sperm hyperactivation increases by about 50% when a high-conductance, K^+-selective channel is inhibited by 4-aminopyridine (Gu et al., 2004). These results illustrate a level of inconsistency concerning the function and identification of K^+ channels in mammalian spermatozoa, confusion that may have arisen from differences in incubation conditions, state of capacitation, et cetera. In addition, one also has to be careful when interpreting the data for ion channels since some early studies involve the characterization of channels in germ cells such as round spermatids and the extrapolation of these results to sperm, an approach that may be not be valid for certain channels.

Recently, advances in patch-clamping, which enables direct measurements in sperm, and the genetic deletion of the *Kcnu1* gene have helped to clarify the situation. When *Kcnu1* is ablated, all pH-dependent K^+ current at physiological membrane potentials is abolished in corpus or caudal epididymal sperm (Santi et al., 2010; Zeng et al., 2011). The male mice harboring this deletion are sterile and, instead of undergoing hyperpolarization under capacitating conditions, the sperm become depolarized. Furthermore, the *Kcnu1*-null sperm show impaired motility and a propensity for flagellar angularity, whereby the sperm tail bends back into a “hairpin” shape (Santi et al., 2010; Zeng et al., 2011). The mutant sperm fail to undergo acrosomal exocytosis, but this defect can be rescued with valinomycin, which hyperpolarizes the *Kcnu1*-null sperm (Santi et al., 2010). These results provide strong support for KCNU1 being the principal potassium channel of mouse sperm with a role in the capacitation-related hyperpolarization, a critical factor for acrosomal exocytosis.

Sodium and protons

How protons and sodium ions factor into the calcium and potassium ion changes in sperm is another extremely important area of interest among sperm physiologists. In human sperm, Lishko et al. (2010) have recently identified the proton channel Hv1 as the predominant effector of proton extrusion. This protein is localized in the principal piece of human sperm. The outward transport of protons is activated by membrane depolarization, and is dependent on an alkaline extracellular environment. The endocannabinoid anandamide activates Hv1 whereas extracellular zinc is a potent inhibitor. Sperm also rely upon Na,K-ATPase α4 for fertility; mouse sperm lacking this flagellar protein are incapable of fertilizing eggs in vitro and the knockout males are infertile (Jimenez et al., 2011). The sperm from the mutant mice have decreased motility and hyperactivation, and exhibit a bend of their flagella (angulation) that has been seen in sperm from other knockout mice such as *Sacy*$^{-/-}$ and *Kcnu*$^{-/-}$ males (Esposito et al., 2004; Hess et al., 2005; Santi et al., 2010; Zeng et al., 2011). Flagellar angulation is a symptom of ionic imbalances within the knockout sperm, which is confirmed by the higher intracellular Na^+ levels in the sperm deficient in Na,K-ATPase α4 (Jimenez et al., 2011).

The sperm sodium-hydrogen exchanger (SLC9A10; previously known as sNHE) is a cation-proton anti-porter that may participate in the alkalinization of sperm during capacitation (Wang et al., 2007). Immunohistochemisty localizes the SLC9A10in the principal piece (Wang et al., 2003). Male mice null for the *Slc9a10* gene are infertile due to aberrant sperm motility, although general development and spermatogenesis are otherwise normal. Sperm motility is almost completely rescued when a cAMP analog is added to the medium, indicating that SLC9A10 function may be involved in the cAMP signaling pathway (Wang et al., 2003). Co-expression of recombinant SACY and SLC9A10 in somatic cells can be co-

immunoprecipitated, suggesting that they are capable of association with each other (Wang et al., 2007), but this result conflicts with published evidence demonstrating that these two proteins are found in separate sperm compartments (midpiece and principal piece, respectively) (Hess et al., 2005; Wang et al., 2003). If SACY and SLC9A10 can be demonstrated to co-localize in sperm, this would provide a direct link for the modulation of intracellular pH and cAMP levels.

Bicarbonate and chloride

Chloride channels are essential for sperm capacitation (Wertheimer et al., 2008; Chen et al., 2009). The absence of Cl^- in the medium does not affect sperm viability, but capacitation-associated processes such as the increase in protein tyrosine phosphorylation, the increase in cAMP levels, hyperactivation, ZP-induced acrosomal exocytosis, and, most importantly, fertilization are abolished or significantly reduced when chloride is replaced by gluconate (Wertheimer et al., 2008). Implicated in this pathway is the cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-gated chloride and bicarbonate channel present in human and mouse sperm (Chan et al., 2006; Xu et al., 2007). CFTR inhibitors or antibodies block HCO_3^--dependent events, such as the increases in intracellular pH, cAMP production, and membrane hyperpolarization. In mouse sperm, capacitation-associated membrane hyperpolarization is regulated by a cAMP/protein kinase A-dependent pathway involving activation of K_{ir} channels, a HCO_3^--sensitive Na/H exchanger, and inhibition of epithelial sodium channels (ENaCs) (Demarco et al., 2003; Hernández-González et al., 2006; Acevedo et al., 2006). Cyclic AMP-dependent Cl^- fluxes through CFTR regulate ENaC during capacitation, and thus contribute to the observed hyperpolarization associated with this process (Hernández-González et al., 2007). A Cl^-/HCO_3^- exchanger (SLC26A) is also present in sperm, but its function is not clear (Chen et al., 2009).

Activation during fertilization: Acquisition of ability to undergo acrosomal exocytosis

Acrosomal exocytosis - a special type of controlled exocytosis

One way that capacitation prepares sperm for fertilization is by priming the cells for acrosomal exocytosis, an event that is stimulated by the ZP of the egg or by progesterone. Acrosomal exocytosis, an absolute prerequisite for fertilization, differs from most other exocytotic systems: 1) each sperm has a only one secretory vesicle, the acrosome; 2) multiple fusion points form between the outer acrosomal membrane and the overlying plasma membrane; 3) membrane loss from the sperm occurs when the outer acrosomal membrane and plasma membrane fuse to form hybrid membrane vesicles and particles that are shed during exocytosis; and 4) as a consequence, membrane recycling does not take place, making acrosomal exocytosis irreversible (Mayorga et al., 2007). Over many years, several physiological events of the capacitation process have been dissected out, including changes in swimming patterns, protein localization changes, increases in membrane fluidity, cholesterol efflux, intracellular Ca^{2+} and cAMP concentration changes, alterations in protein tyrosine phosphorylation, and polymerization of actin (F-actin formation) in the sperm head (Brener et al., 2003; Finkelstein et al., 2010). Yet, the question that has remained unanswered is: What are the underlying steps that transform the sperm cell from a state unresponsive to ZP- or progesterone-stimulated acrosomal exocytosis to one primed to respond to these stimuli?

Changes from a non-responsive cell to a responsive cell

Acrosomal exocytosis is not an all-or-none event in which the acrosome is either “intact” or “reacted”. Furthermore, sperm may undergo acrosomal exocytosis spontaneously or in

response to a stimulus such as ZP proteins or progesterone. “Spontaneous” acrosomal exocytosis can either be interpreted as an aberrant process that is irrelevant or it can be considered a physiologically meaningful event. We favor the latter view, and envision that capacitation prepares sperm for the initiation of exocytosis; in some cases, the sperm is primed like a “hair trigger” wherein the slightest perturbation can “fire off” the acrosome prematurely (i.e., spontaneously). For instance, the natural accumulation of signals with the sperm over the course of capacitation may exceed a threshold, causing exocytosis to occur (Kim and Gerton, 2003). In most cases, however, the exocytotic event results from stimulation by some external factor such as ZP proteins or progesterone. The distinction between the term stimulation and induction of acrosomal exocytosis is more than semantic. The term “stimulation” implies a continuously variable (analog) rise in a signal whereas “induction” suggests an off/on (binary) state, much like flipping a switch to trigger an event. Our view is that acrosomal exocytosis is initiated during capacitation, and progresses through several intermediate stages before secretion of the acrosomal contents is completed (Kim and Gerton, 2003; Buffone et al., 2008).

When sperm are incubated under capacitating conditions, they spontaneously but asynchronously progress through defined morphologically and biochemically intermediate stages of acrosomal exocytosis that lead to the incremental exposure and eventual release of acrosomal components, the rate of which is dependent upon their state within the acrosome: soluble or particulate (acrosomal matrix-associated). For example, multiple intermediate stages of spontaneous human sperm acrosomal exocytosis can be identified by transmission electron microscopy (Stock and Fraser, 1987; Yudin et al., 1988). Antibodies to acrosomal matrix components have been used to define successive transitional stages leading to the complete release of acrosomal components in sperm from transgenic mice that carry soluble green fluorescent protein (GFP) in their acrosomes (Kim and Gerton, 2003). Four stages of exocytosis represent acrosomes that are intact, acrosomes that have exposed internal proteins (ZP3R/sp56) but have not released soluble components (GFP), acrosomes that have released soluble GFP but still retain acrosomal matrix ZP3R, and acrosomes that have lost both the soluble GFP and matrix ZP3R.

Capacitated sperm are poised for exocytosis, and treatment of these cells with a stimulant such as ZP proteins, follicular fluid, or progesterone speeds up the process, leading to a more rapid and universal release of acrosomal contents and resulting in a more synchronous secretory event. In these situations, multiple intermediate stages of acrosomal exocytosis following follicular fluid treatment can also be identified by transmission electron microscopy (Stock and Fraser, 1987; Yudin et al., 1988). An intermediate stage of mouse acrosomal exocytosis was also identified by chlortetracycline fluorescence of sperm treated with ZP proteins from 12-*O*-tetradecanoyl phorbol-13-acetate-treated oocytes (Kligman et al., 1991). In guinea pig sperm stimulated to undergo acrosomal exocytosis with the calcium ionophore A23187, antibodies to acrosomal components demonstrated that the transitional release of acrosomal proteins depended on their relationship with the acrosomal matrix, where soluble proteins were more readily released than matrix components and where different matrix components exhibited different rates of release, leading to the complete release of acrosomal components (Kim and Gerton, 2003). More recently, Zanetti and Mayorga (2009) demonstrated the existence of intermediate morphological steps of hybrid vesicle formation prior to complete acrosomal exocytosis in human sperm. They reported that during capacitation, acrosomal swelling causes changes in distance between the outer acrosomal membrane and the plasma membrane, and that the probable contact between these two membranes could establish acrosome docking or the formation of fusion pores. The formation of these intermediate stages is coincident with stabilization of a primed event (membrane docking/fusion) so that the exocytosis is restricted and occurs more rapidly in response to a specific stimulus (e.g., ZP or progesterone).

The actin cytoskeleton plays an important role in regulating the process of acrosomal exocytosis. Although different species show variable patterns, actin has been localized between the plasma membrane and outer acrosomal membrane along the concave margin of the hamster sperm head; in the perinuclear space underlying the postacrosomal sheath of bull and rabbit sperm; around the connecting piece in the neck region of sperm from all four species; and on the external surface of the fibrous sheath of human sperm (Flaherty et al., 1988). In mammalian sperm, actin is present in its monomeric, globular form (G-actin) as well as filamentous actin (F-actin). During the course of capacitation, G-actin is polymerized to F-actin (Cabello-Agüeros et al., 2003; Castellani-Ceresa et al., 1993; Brener et al., 2003; Finkelstein et al., 2010). In somatic systems, the cortical filamentous actin network acts as a dominant-negative clamp that blocks constitutive exocytosis (Muallem et al., 1995). Using a two-photon excitation imaging system, Nemoto and coworkers (2004) revealed that priming of zymogen granules for fusion in pancreatic acinar cells is accompanied by the rapid coating with F-actin, which serves to slow the rate of granule fusion without reducing the overall extent of exocytosis whereas latrunculin A, an inhibitor of actin polymerization, reduced the latency to exocytosis. Inhibition of actin depolymerization by phalloidin in a permeabilized sperm model inhibits acrosomal exocytosis, indicating that the dispersion of F-actin is necessary for acrosomal exocytosis to occur (Hernández-González et al., 2000). Thus, F-actin stabilizes the exocytotic machinery to restrict secretory granules from fusing with the plasma membrane. When capacitated sperm are stimulated to undergo acrosomal exocytosis, rapid F-actin depolymerization occurs (Spungin et al., 1995). Moreover, inhibition of actin polymerization blocks ZP-induced acrosomal exocytosis and sperm penetration into zona-free eggs, thus interfering with the ability of boar and mouse sperm to become competent for in vitro fertilization (Liu et al., 1999; Rogers et al., 1989; Castellani-Ceresa et al., 1993; Brener et al., 2003). Activation of PKA using cAMP analogues, as well as activation of PKC using phorbol esters, significantly enhances actin polymerization in bovine sperm (Brener et al., 2003; Finkelstein et al., 2010). A rise in intracellular calcium appears to be a key event in the depolymerization of actin, resulting in acrosomal exocytosis (Spungin and Breitbart, 1996). This appears to function, in part, through the activation of the actin severing protein, gelsolin (Finkelstein et al., 2010).

Molecular events during acrosomal exocytosis

Calcium is obligatory for acrosomal exocytosis and comes from extracellular and intracellular sources (Florman and Ducibella, 2006). One theory postulates that the acrosome is a calcium store and that depletion of Ca^{2+} from the acrosome activates store-operated channels that allow sustained entry of Ca^{2+} from the medium (Herrick et al., 2005). On the other hand, others have demonstrated that the Ca^{2+} rise induced by exposure to the ZP or progesterone started at different sites within the sperm head, indicating that these agonists may stimulate acrosomal exocytosis via different mechanisms (Meizel et al., 1997; Fukami et al., 2003). Ho and Suarez (2003) identified the redundant nuclear envelope at the posterior end of the sperm head as a calcium store. Along these lines, Xia and Ren (2009) have recently captured intriguing video images with Fluo-4 that indicate the ZP-induced Ca^{2+} increase starts in the sperm tail (near the annulus) and propagates toward the head (anterograde direction). Using calcium-imaging studies, the anterograde wave of calcium in response to soluble ZP suggests that a calcium store near the head-tail junction may be associated with ZP-stimulated acrosomal exocytosis (Ho and Suarez, 2003; Costello et al., 2009). The direction of a Ca^{2+} wave progressing from the base of the head toward the apical tip is reminiscent of our studies demonstrating the anterograde loss of acrosomal GFP from transgenic mouse sperm exposed to soluble zona proteins (Buffone et al., 2009).

SNARE proteins and synaptotagmin are involved in the final stages of fusion of the outer acrosomal membrane and plasma membrane, but there is no recognized mechanism for the membrane movements that precede the point fusions (Burgoyne and Morgan, 2003; De Blas et al., 2005; Mayorga et al., 2007). Branham and coworkers (Branham et al., 2006, 2009), have shown that cAMP, via EPAC activation, has the ability to trigger the complete set of events necessary to achieve complete acrosomal exocytosis, including tethering and docking of the acrosome to the plasma membrane, priming of the fusion machinery, mobilization of Ca^{2+}, and fusion of the outer acrosomal and the plasma membranes.

Exciting new findings challenge existing paradigms

In this brief review, we have emphasized the three themes of compartmentalization, competence, and commitment as features of sperm activation. Compartmentalization serves to place distinct functions in different regions of the sperm. The downside of this type of arrangement is that the control of processes must be local, unless there exists a mechanism for communication between compartments. Such a mechanism could operate at the plasma membrane level or within the cytoplasm of the sperm. In either event, ion flux could be involved as a pathway for the relatively rapid propagation of a signal. The competence of a given sperm to acquire the ability for forward motility in the cauda epididymis, to display hyperactivated motility in the female reproductive tract, or to become primed for acrosomal exocytosis is gained in specific places and at particular periods during the lifetime of a sperm. The commitment of a sperm to hyperactivated motility or acrosomal exocytosis is dependent upon the environmental milieu and other factors.

We have been intrigued in recent years with several observations that did not fit in the generally accepted paradigm for events of sperm physiology. We mentioned a few of these above. One finding from our own laboratory was that soluble zona proteins specifically stimulate the loss of acrosomal GFP in an anterograde direction whereas the calcium ionophore ionomycin, which would be expected to function all over the sperm surface, induces the GFP release at random sites along the acrosomal cap (Buffone et al., 2009). This suggests that the soluble zona proteins work through a regionally localized (i.e., compartmentalized) receptor. This idea is strongly supported by calcium imaging results demonstrating that soluble zona proteins cause an influx into or a release of free calcium starting in the sperm tail and progressing in an anterograde direction (Xia and Ren, 2009). Other workers have also demonstrated a wave of calcium in response to compounds such as progesterone and zona proteins (Meizel et al., 1997; Fukami et al., 2003).

It is also striking and important to recognize that the sperm plasma itself is indeed compartmentalized. A variety of transmembrane or glycophosphatidyl inositide-linked plasma membrane proteins have very discrete localizations on the sperm surface. Curiously, proteins such as basigin of rat and mouse sperm and PT-1 of guinea pig sperm start out being confined to the principal piece of the flagellum even though they are freely diffusible within this region (Cesario and Bartles, 1994; Myles et al., 1984). Under conditions of epididymal maturation (basigin) or capacitation (PT-1), these proteins then move into the midpiece of the flagellum. Although the functions of these proteins in sperm are unknown, it is now recognized that the annulus of the sperm flagellum is required to establish a membrane diffusion barrier between the midpiece and principal piece, apparently involving septins associated with the annulus structure (Kwitny et al., 2010). This is a key finding since septins also establish a barrier to the diffusion of membrane proteins at the base of the primary cilium of somatic cells; when septin 2 is depleted, membrane protein localization is disrupted and Sonic hedgehog signaling is lost (Hu et al., 2010). It remains to be seen if the septins associated with the annulus restrain specific signaling molecules such as receptors within the principal piece of the sperm flagellum.

Ion channels in the sperm plasma membrane are also compartmentalized. The Hv1 proton pump, involved in the alkalinization of the sperm cytoplasm, is compartmentalized within the principal piece (Lishko et al., 2010). The CATSPER proteins are also found in the principal piece of the sperm tail (Ren et al., 2001). It is also likely that the KCNU1 (Slo3/K_{sper}) channel is located in the principal piece since this would be consistent with the electrophysiology (Navarro et al., 2007). Both CATSPER and KCNU1 are activated by alkalinization (Schreiber et al., 1998; Zhang et al., 2006; Kirichok et al., 2006). Recently, CATSPER has been identified as the mediator of the progesterone-induced Ca^{2+} influx in human sperm (Lishko et al., 2011; Strunker et al., 2011).

Another finding that has been observed by multiple laboratories concerns the binding of transgenic mouse sperm expressing enhanced GFP (EGFP) in their acrosomes to the particulate ZP surrounding oocytes. These sperm have been observed to bind to the zonae pellucidae of unfertilized eggs, and to remain there in an ”intact” or EGFP-positive state for an extended period of time without loss of the EGFP (Nakanishi et al., 1999; Baibakov et al., 2007). Challenging the concept that sperm must be “acrosome-intact” to bind to the zona is recent work from Hirohashi and colleagues. Using real-time imaging of the fertilization of cumulus-enclosed oocytes (which more closely resembles the in vitro situation in the mouse than do denuded oocytes), these investigators found that most fertilizing spermatozoa had begun acrosomal exocytosis before reaching and penetrating penetrate the zona (Jin et al., 2011). As judged by the presence of peanut agglutinin reactivity following loss of the soluble EGFP from the fertilizing sperm in the cumulus mass, the membranes over the acrosome are lost before the sperm encounter the ZP; however, peanut agglutinin-reactive, particulate acrosomal matrix material that likely is involved in sperm-zona binding is still present (Hirohashi et al., 2011).

From these experiments, it appears that the acrosomal region contains proteins capable of binding the head of the sperm to the ZP, but that the stimulation of exocytosis (as detected by the loss of acrosomal EGFP) may actually start with flagellar response to a signal in the extracellular milieu. Figure 3 illustrates a speculative model whereby sensing molecules (i.e., a signaling receptor for zona proteins or progesterone) could be present in the flagellar plasma membrane, and could operate much in the same way that sensory cilia do in somatic cells (Bloodgood, 2010). In response to binding a factor in the cumulus extracellular matrix/fluid, the resulting calcium signal could propagate toward the head and stimulate exocytosis (Fig. 3). Cumulus-intact oocytes have higher fertilization rates than cumulus-free eggs, and the cumulus extracellular fluid also contains progesterone, a known stimulant of acrosomal exocytosis and reported chemoattractant (Oren-Benaroya et al., 2008). Because cumulus cells envelope the oocyte during the biogenesis of the ZP, there is also the possibility that soluble ZP proteins or fragments thereof that do not polymerize into the final zona structure diffuse into the extracellular matrix of the cumulus cells and can function as ligands for receptors on the sperm surface (possibly involving the principal piece of the flagellum).

Clearly, this is an exciting time for the field of sperm biology. Although there are many, many questions to be answered concerning the molecular mechanisms of sperm activation, there have been some very exciting and thought-provoking findings that have occurred in recent years. We must, however, look beyond our reductionist experimental approaches and bear in mind what is the natural situation for sperm. They really never experience an environment akin to the simple buffers we use in the laboratory; indeed, they are naturally found in a much more viscous milieu that is abundant in proteins and bioactive molecules. The challenge for us is to broaden our thinking beyond the currently accepted paradigms, and to continue to develop innovative ways to discover the roles of the various ion channels, enzymes, second messenger signaling molecules, metabolites, and environmental conditions so that we may improve our understanding of the ways that functional compartmentalization

affects the competence of sperm and the ability of these cells to commit to the final steps of fertilization.

Acknowledgments

Grant sponsor: National Institutes of Health. **Grant numbers**: HD-041552, HD-057144, and HD-051999 (to GLG).

Abbreviations

cAMP	cyclic adenosine monophosphate
CATSPER	cation channel, sperm associated
[E]GFP	[enhanced] green fluorescent protein
EPAC	exchange proteins activated by cAMP
PKA	protein kinase A
ZP	zona pellucida

References

Acevedo JJ, Mendoza-Lujambio I, de la Vega-Beltrán JL, Treviño CL, Felix R, Darszon A. KATP channels in mouse spermatogenic cells and sperm, and their role in capacitation. Dev. Biol. 2006; 289:395–405. [PubMed: 16343479]

Aparicio IM, Bragado MJ, Gil MC, Garcia-Herreros M, Gonzalez-Fernandez L, Tapia JA, Garcia-Marin LJ. Porcine sperm motility is regulated by serine phosphorylation of the glycogen synthase kinase-3alpha. Reproduction. 2007; 134:435–444. [PubMed: 17709562]

Arnoult C, Cardullo RA, Lemos JR, Florman HM. Activation of mouse sperm T-type Ca2+ channels by adhesion to the egg zona pellucida. Proc. Natl. Acad. Sci. U.S.A. 1996; 93:13004–13009. [PubMed: 8917534]

Avenarius MR, Hildebrand MS, Zhang Y, Meyer NC, Smith LLH, Kahrizi K, Najmabadi H, Smith RJH. Human male infertility caused by mutations in the CATSPER1 channel protein. Am. J. Hum. Genet. 2009; 84:505–510. [PubMed: 19344877]

Baibakov B, Gauthier L, Talbot P, Rankin TL, Dean J. Sperm binding to the zona pellucida is not sufficient to induce acrosome exocytosis. Development. 2007; 134:933–943. [PubMed: 17293534]

Baker MA, Witherdin R, Hetherington L, Cunningham-Smith K, Aitken RJ. Identification of post-translational modifications that occur during sperm maturation using difference in two-dimensional gel electrophoresis. Proteomics. 2005; 5:1003–1012. [PubMed: 15712234]

Baxendale RW, Fraser LR. Evidence for multiple distinctly localized adenylyl cyclase isoforms in mammalian spermatozoa. Mol. Reprod. Dev. 2003; 66:181–189. [PubMed: 12950106]

De Blas GA, Roggero CM, Tomes CN, Mayorga LS. Dynamics of SNARE assembly and disassembly during sperm acrosomal exocytosis. PLoS Biol. 2005; 3:e323. [PubMed: 16131227]

Bloodgood RA. Sensory reception is an attribute of both primary cilia and motile cilia. J Cell Sci. 2010; 123:505–509. [PubMed: 20144998]

Branham MT, Bustos MA, De Blas GA, Rehmann H, Zarelli VEP, Treviño CL, Darszon A, Mayorga LS, Tomes CN. Epac activates the small G proteins Rap1 and Rab3A to achieve exocytosis. J. Biol. Chem. 2009; 284:24825–24839. [PubMed: 19546222]

Branham MT, Mayorga LS, Tomes CN. Calcium-induced acrosomal exocytosis requires cAMP acting through a protein kinase A-independent, Epac-mediated pathway. J. Biol. Chem. 2006; 281:8656–8666. [PubMed: 16407249]

Brener E, Rubinstein S, Cohen G, Shternall K, Rivlin J, Breitbart H. Remodeling of the actin cytoskeleton during mammalian sperm capacitation and acrosome reaction. Biol. Reprod. 2003; 68:837–845. [PubMed: 12604633]

Buffone MG, Foster JA, Gerton GL. The role of the acrosomal matrix in fertilization. Int. J. Dev. Biol. 2008; 52:511–522. [PubMed: 18649264]

Buffone MG, Rodriguez-Miranda E, Storey BT, Gerton GL. Acrosomal exocytosis of mouse sperm progresses in a consistent direction in response to zona pellucida. J. Cell. Physiol. 2009; 220:611–620. [PubMed: 19373867]

Burgoyne RD, Morgan A. Secretory granule exocytosis. Physiol. Rev. 2003; 83:581–632. [PubMed: 12663867]

Burton KA, Treash-Osio B, Muller CH, Dunphy EL, McKnight GS. Deletion of type IIalpha regulatory subunit delocalizes protein kinase A in mouse sperm without affecting motility or fertilization. J. Biol. Chem. 1999; 274:24131–24136. [PubMed: 10446185]

Cabello-Agüeros JF, Hernández-González EO, Mújica A. The role of F-actin cytoskeleton-associated gelsolin in the guinea pig capacitation and acrosome reaction. Cell Motil. Cytoskeleton. 2003; 56:94–108. [PubMed: 14506707]

Calvin HI, Bedford JM. Formation of disulphide bonds in the nucleus and accessory structures of mammalian spermatozoa during maturation in the epididymis. J. Reprod. Fertil. Suppl. 1971; 13(Suppl 13):65–75. [PubMed: 5292801]

Carlson AE, Quill TA, Westenbroek RE, Schuh SM, Hille B, Babcock DF. Identical phenotypes of CatSper1 and CatSper2 null sperm. J. Biol. Chem. 2005; 280:32238–32244. [PubMed: 16036917]

Carrera A, Gerton GL, Moss SB. The major fibrous sheath polypeptide of mouse sperm: structural and functional similarities to the A-kinase anchoring proteins. Dev. Biol. 1994; 165:272–284. [PubMed: 8088444]

Castellani-Ceresa L, Mattioli M, Radaelli G, Barboni B, Brivio MF. Actin polymerization in boar spermatozoa: fertilization is reduced with use of cytochalasin D. Mol. Reprod. Dev. 1993; 36:203–211. [PubMed: 8257569]

Cesario MM, Bartles JR. Compartmentalization, processing and redistribution of the plasma membrane protein CE9 on rodent spermatozoa. Relationship of the annulus to domain boundaries in the plasma membrane of the tail. J. Cell. Sci. 1994; 107(Pt 2):561–570. [PubMed: 8207079]

Chan HC, Shi QX, Zhou CX, Wang XF, Xu WM, Chen WY, Chen AJ, Ni Y, Yuan YY. Critical role of CFTR in uterine bicarbonate secretion and the fertilizing capacity of sperm. Mol. Cell. Endocrinol. 2006; 250:106–113. [PubMed: 16414184]

Chen WY, Xu WM, Chen ZH, Ni Y, Yuan YY, Zhou SC, Zhou WW, Tsang LL, Chung YW, Höglund P, Chan HC, Shi QX. Cl− is required for HCO3− entry necessary for sperm capacitation in guinea pig: Involvement of a Cl−/HCO3− exchanger (SLC26A3) and CFTR. Biol. Reprod. 2009; 80:115–123. [PubMed: 18784352]

Cobellis G, Ricci G, Cacciola G, Orlando P, Petrosino S, Cascio MG, Bisogno T, De Petrocellis L, Chioccarelli T, Altucci L, Fasano S, Meccariello R, Pierantoni R, Ledent C, Di Marzo V. A gradient of 2-arachidonoylglycerol regulates mouse epididymal sperm cell start-up. Biol. Reprod. 2010; 82:451–458. [PubMed: 19812302]

Cornwall GA, Vindivich D, Tillman S, Chang TS. The effect of sulfhydryl oxidation on the morphology of immature hamster epididymal spermatozoa induced to acquire motility in vitro. Biol. Reprod. 1988; 39:141–155. [PubMed: 3207793]

Cornwall GA. New insights into epididymal biology and function. Hum. Reprod. Update. 2009; 15:213–227. [PubMed: 19136456]

Costello S, Michelangeli F, Nash K, Lefievre L, Morris J, Machado-Oliveira G, Barratt C, Kirkman-Brown J, Publicover S. Ca2+-stores in sperm: their identities and functions. Reproduction. 2009; 138:425–437. [PubMed: 19542252]

Dacheux JL, Belghazi M, Lanson Y, Dacheux F. Human epididymal secretome and proteome. Mol. Cell. Endocrinol. 2006; 250:36–42. [PubMed: 16431015]

Dacheux J-L, Gatti JL, Dacheux F. Contribution of epididymal secretory proteins for spermatozoa maturation. Microsc. Res. Tech. 2003; 61:7–17. [PubMed: 12672118]

Danshina PV, Geyer CB, Dai Q, Goulding EH, Willis WD, Kitto GB, McCarrey JR, Eddy EM, O'Brien DA. Phosphoglycerate kinase 2 (PGK2) is essential for sperm function and male fertility in mice. Biol. Reprod. 2010; 82:136–145. [PubMed: 19759366]

Demarco IA, Espinosa F, Edwards J, Sosnik J, de la Vega-Beltrán JL, Hockensmith JW, Kopf GS, Darszon A, Visconti PE. Involvement of a Na+/HCO-3 cotransporter in mouse sperm capacitation. J. Biol. Chem. 2003; 278:7001–7009. [PubMed: 12496293]

Eddy, EM. Chapter 1. The Spermatozoon. In: Knobil, E., editor. Knobil and Neill's physiology of reproduction,3e. Elsevier; Amsterdam: Boston: 2006. p. 3-54.

Escoffier J, Boisseau S, Serres C, Chen C-C, Kim D, Stamboulian S, Shin H-S, Campbell KP, De Waard M, Arnoult C. Expression, localization and functions in acrosome reaction and sperm motility of Ca(V)3.1 and Ca(V)3.2 channels in sperm cells: an evaluation from Ca(V)3.1 and Ca(V)3.2 deficient mice. J. Cell. Physiol. 2007; 212:753–763. [PubMed: 17450521]

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. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101:2993–2998. [PubMed: 14976244]

Finkelstein M, Etkovitz N, Breitbart H. Role and Regulation of Sperm Gelsolin Prior to Fertilization. Journal of Biological Chemistry. 2010; 285:39702–39709. [PubMed: 20937821]

Flaherty SP, Winfrey VP, Olson GE. Localization of actin in human, bull, rabbit, and hamster sperm by immunoelectron microscopy. Anat. Rec. 1988; 221:599–610. [PubMed: 3414983]

Florman, HM.; Ducibella, T. Fertilization in Mammals. In: Knobil, E.; Neill, editors. Knobil and Neill's physiology of reproduction,3e. Elsevier; Amsterdam: Boston: 2006. p. 55-112.

Fukami K, Yoshida M, Inoue T, Kurokawa M, Fissore RA, Yoshida N, Mikoshiba K, Takenawa T. Phospholipase Cdelta4 is required for Ca2+ mobilization essential for acrosome reaction in sperm. J. Cell Biol. 2003; 161:79–88. [PubMed: 12695499]

Fulcher KD, Mori C, Welch JE, O'Brien DA, Klapper DG, Eddy EM. Characterization of Fsc1 cDNA for a mouse sperm fibrous sheath component. Biol. Reprod. 1995; 52:41–49. [PubMed: 7711182]

Glassner M, Jones J, Kligman I, Woolkalis MJ, Gerton GL, Kopf GS. Immunocytochemical and biochemical characterization of guanine nucleotide-binding regulatory proteins in mammalian spermatozoa. Dev. Biol. 1991; 146:438–450. [PubMed: 1907582]

Gu Y, Kirkman-Brown JC, Korchev Y, Barratt CLR, Publicover SJ. Multi-state, 4-aminopyridine-sensitive ion channels in human spermatozoa. Dev. Biol. 2004; 274:308–317. [PubMed: 15385161]

Hernández-González EO, Lecona-Valera AN, Escobar-Herrera J, Mújica A. Involvement of an F-actin skeleton on the acrosome reaction in guinea pig spermatozoa. Cell Motil. Cytoskeleton. 2000; 46:43–58. [PubMed: 10842332]

Hernández-González EO, Sosnik J, Edwards J, Acevedo JJ, Mendoza-Lujambio I, López-González I, Demarco I, Wertheimer E, Darszon A, Visconti PE. Sodium and epithelial sodium channels participate in the regulation of the capacitation-associated hyperpolarization in mouse sperm. J. Biol. Chem. 2006; 281:5623–5633. [PubMed: 16407190]

Hernández-González EO, Treviño CL, Castellano LE, de la Vega-Beltrán JL, Ocampo AY, Wertheimer E, Visconti PE, Darszon A. Involvement of cystic fibrosis transmembrane conductance regulator in mouse sperm capacitation. J. Biol. Chem. 2007; 282:24397–24406. [PubMed: 17588945]

Herrick SB, Schweissinger DL, Kim S-W, Bayan KR, Mann S, Cardullo RA. The acrosomal vesicle of mouse sperm is a calcium store. J. Cell. Physiol. 2005; 202:663–671. [PubMed: 15389568]

Hess KC, Jones BH, Marquez B, Chen Y, Ord TS, Kamenetsky M, Miyamoto C, Zippin JH, Kopf GS, Suarez SS, Levin LR, Williams CJ, Buck J, Moss SB. The "soluble" adenylyl cyclase in sperm mediates multiple signaling events required for fertilization. Developmental Cell. 2005; 9:249–259. [PubMed: 16054031]

Hirohashi N, Gerton GL, Buffone MG. Video imaging of the sperm acrosome reaction during in vitro fertilization. Comm. Integ. Biol. 2011; 4:471–476.

Ho H-C, Suarez SS. Characterization of the intracellular calcium store at the base of the sperm flagellum that regulates hyperactivated motility. Biol. Reprod. 2003; 68:1590–1596. [PubMed: 12606347]

Ho K, Wolff CA, Suarez SS. CatSper-null mutant spermatozoa are unable to ascend beyond the oviductal reservoir. Reprod. Fertil. Dev. 2009; 21:345–350. [PubMed: 19210926]

Hu Q, Milenkovic L, Jin H, Scott MP, Nachury MV, Spiliotis ET, Nelson WJ. A septin diffusion barrier at the base of the primary cilium maintains ciliary membrane protein distribution. Science. 2010; 329:436–439. [PubMed: 20558667]

Jimenez T, Sanchez G, McDermott JP, Nguyen A-N, Kumar TR, Blanco G. Increased expression of the Na,K-ATPase alpha4 isoform enhances sperm motility in transgenic mice. Biol. Reprod. 2011; 84:153–161. [PubMed: 20826726]

Jin J, Jin N, Zheng H, Ro S, Tafolla D, Sanders KM, Yan W. Catsper3 and Catsper4 are essential for sperm hyperactivated motility and male fertility in the mouse. Biol. Reprod. 2007; 77:37–44. [PubMed: 17344468]

Jin M, Fujiwara E, Kakiuchi Y, Okabe M, Satouh Y, Baba SA, Chiba K, Hirohashi N. Most fertilizing mouse spermatozoa begin their acrosome reaction before contact with the zona pellucida during in vitro fertilization. Proceedings of the National Academy of Sciences. 2011; 108:4892–4896.

Kamenetsky M, Middelhaufe S, Bank EM, Levin LR, Buck J, Steegborn C. Molecular details of cAMP generation in mammalian cells: a tale of two systems. J. Mol. Biol. 2006; 362:623–639. [PubMed: 16934836]

Kim K-S, Gerton GL. Differential release of soluble and matrix components: evidence for intermediate states of secretion during spontaneous acrosomal exocytosis in mouse sperm. Dev. Biol. 2003; 264:141–152. [PubMed: 14623237]

Kirichok Y, Navarro B, Clapham DE. Whole-cell patch-clamp measurements of spermatozoa reveal an alkaline-activated Ca2+ channel. Nature. 2006; 439:737–740. [PubMed: 16467839]

Kligman I, Glassner M, Storey BT, Kopf GS. Zona pellucida-mediated acrosomal exocytosis in mouse spermatozoa: Characterization of an intermediate stage prior to the completion of the acrosome reaction. Dev. Biol. 1991; 145:344–355. [PubMed: 2040376]

Kwitny S, Klaus AV, Hunnicutt GR. The annulus of the mouse sperm tail is required to establish a membrane diffusion barrier that is engaged during the late steps of spermiogenesis. Biol. Reprod. 2010; 82:669–678. [PubMed: 20042538]

Lishko PV, Botchkina IL, Fedorenko A, Kirichok Y. Acid extrusion from human spermatozoa is mediated by flagellar voltage-gated proton channel. Cell. 2010; 140:327–337. [PubMed: 20144758]

Lishko PV, Botchkina IL, Kirichok Y. Progesterone activates the principal Ca2+ channel of human sperm. Nature. 2011; 471:387–391. [PubMed: 21412339]

Liu DY, Martic M, Clarke GN, Dunlop ME, Baker HW. An important role of actin polymerization in the human zona pellucida-induced acrosome reaction. Mol. Hum. Reprod. 1999; 5:941–949. [PubMed: 10508222]

Livera G, Xie F, Garcia MA, Jaiswal B, Chen J, Law E, Storm DR, Conti M. Inactivation of the mouse adenylyl cyclase 3 gene disrupts male fertility and spermatozoon function. Mol. Endocrinol. 2005; 19:1277–1290. [PubMed: 15705663]

Lobley A, Pierron V, Reynolds L, Allen L, Michalovich D. Identification of human and mouse CatSper3 and CatSper4 genes: characterisation of a common interaction domain and evidence for expression in testis. Reprod. Biol. Endocrinol. 2003; 1:53. [PubMed: 12932298]

Machado-Oliveira G, Lefievre L, Ford C, Herrero MB, Barratt C, Connolly TJ, Nash K, Morales-Garcia A, Kirkman-Brown J, Publicover S. Mobilisation of Ca2+ stores and flagellar regulation in human sperm by S-nitrosylation: a role for NO synthesised in the female reproductive tract. Development. 2008; 135:3677–3686. [PubMed: 18842814]

Marquez B, Suarez SS. Bovine sperm hyperactivation Is promoted by alkaline-stimulated Ca2+ influx. Biol. Reprod. 2007; 76:660–665. [PubMed: 17182893]

Martínez-López P, Santi CM, Treviño CL, Ocampo-Gutiérrez AY, Acevedo JJ, Alisio A, Salkoff LB, Darszon A. Mouse sperm K+ currents stimulated by pH and cAMP possibly coded by Slo3 channels. Biochem. Biophys. Res. Commun. 2009; 381:204–209. [PubMed: 19338774]

Mayorga LS, Tomes CN, Belmonte SA. Acrosomal exocytosis, a special type of regulated secretion. IUBMB Life. 2007; 59:286–292. [PubMed: 17505967]

McCarrey JR, Thomas K. Human testis-specific PGK gene lacks introns and possesses characteristics of a processed gene. Nature. 1987; 326:501–505. [PubMed: 3453121]

Meizel S, Turner KO, Nuccitelli R. Progesterone triggers a wave of increased free calcium during the human sperm acrosome reaction. Dev. Biol. 1997; 182:67–75. [PubMed: 9073447]

Merlet F, Weinstein LS, Goldsmith PK, Rarick T, Hall JL, Bisson JP, de Mazancourt P. Identification and localization of G protein subunits in human spermatozoa. Mol. Hum. Reprod. 1999; 5:38–45. [PubMed: 10050660]

Miki K, Qu W, Goulding EH, Willis WD, Bunch DO, Strader LF, Perreault SD, Eddy EM, O'Brien DA. Glyceraldehyde 3-phosphate dehydrogenase-S, a sperm-specific glycolytic enzyme, is required for sperm motility and male fertility. Proc. Natl. Acad. Sci. U.S.A. 2004; 101:16501–16506. [PubMed: 15546993]

Mori C, Welch JE, Fulcher KD, O'Brien DA, Eddy EM. Unique hexokinase messenger ribonucleic acids lacking the porin-binding domain are developmentally expressed in mouse spermatogenic cells. Biol. Reprod. 1993; 49:191–203. [PubMed: 8396993]

Muallem S, Kwiatkowska K, Xu X, Yin HL. Actin filament disassembly is a sufficient final trigger for exocytosis in nonexcitable cells. J. Cell Biol. 1995; 128:589–598. [PubMed: 7860632]

Myles DG, Primakoff P, Koppel DE. A localized surface protein of guinea pig sperm exhibits free diffusion in its domain. J. Cell Biol. 1984; 98:1905–1909. [PubMed: 6725404]

Nakamura N, Miranda-Vizuete A, Miki K, Mori C, Eddy EM. Cleavage of disulfide bonds in mouse spermatogenic cell-specific type 1 hexokinase isozyme is associated with increased hexokinase activity and initiation of sperm motility. Biol. Reprod. 2008; 79:537–545. [PubMed: 18509164]

Nakanishi T, Ikawa M, Yamada S, Parvinen M, Baba T, Nishimune Y, Okabe M. Real-time observation of acrosomal dispersal from mouse sperm using GFP as a marker protein. FEBS Lett. 1999; 449:277–283. [PubMed: 10338148]

Navarro B, Kirichok Y, Clapham DE. KSper, a pH-sensitive K+ current that controls sperm membrane potential. Proc. Natl. Acad. Sci. U.S.A. 2007; 104:7688–7692. [PubMed: 17460039]

Nemoto T, Kojima T, Oshima A, Bito H, Kasai H. Stabilization of exocytosis by dynamic F-actin coating of zymogen granules in pancreatic acini. J. Biol. Chem. 2004; 279:37544–37550. [PubMed: 15184362]

Nolan MA, Babcock DF, Wennemuth G, Brown W, Burton KA, McKnight GS. Sperm-specific protein kinase A catalytic subunit Calpha2 orchestrates cAMP signaling for male fertility. Proc. Natl. Acad. Sci. U.S.A. 2004; 101:13483–13488. [PubMed: 15340140]

Oren-Benaroya R, Orvieto R, Gakamsky A, Pinchasov M, Eisenbach M. The sperm chemoattractant secreted from human cumulus cells is progesterone. Human Reproduction. 2008; 23:2339–2345. [PubMed: 18621752]

Pastor-Soler N, Beaulieu V, Litvin TN, Da Silva N, Chen Y, Brown D, Buck J, Levin LR, Breton S. Bicarbonate-regulated adenylyl cyclase (sAC) is a sensor that regulates pH-dependent V-ATPase recycling. J. Biol. Chem. 2003; 278:49523–49529. [PubMed: 14512417]

Pinto FM, Ravina CG, Fernández-Sánchez M, Gallardo-Castro M, Cejudo-Román A, Candenas L. Molecular and functional characterization of voltage-gated sodium channels in human sperm. Reprod. Biol. Endocrinol. 2009; 7:71. [PubMed: 19607678]

Qi H, Moran MM, Navarro B, Chong JA, Krapivinsky G, Krapivinsky L, Kirichok Y, Ramsey IS, Quill TA, Clapham DE. All four CatSper ion channel proteins are required for male fertility and sperm cell hyperactivated motility. Proc. Natl. Acad. Sci. U.S.A. 2007; 104:1219–1223. [PubMed: 17227845]

Quill TA, Ren D, Clapham DE, Garbers DL. A voltage-gated ion channel expressed specifically in spermatozoa. Proc. Natl. Acad. Sci. U.S.A. 2001; 98:12527–12531. [PubMed: 11675491]

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; 413:603–609. [PubMed: 11595941]

Rogers BJ, Bastias C, Coulson RL, Russell LD. Cytochalasin D inhibits penetration of hamster eggs by guinea pig and human spermatozoa. J. Androl. 1989; 10:275–282. [PubMed: 2777719]

Da Ros VG, Maldera JA, Willis WD, Cohen DJ, Goulding EH, Gelman DM, Rubinstein M, Eddy EM, Cuasnicu PS. Impaired sperm fertilizing ability in mice lacking Cysteine-RIch Secretory Protein 1 (CRISP1). Dev. Biol. 2008; 320:12–18. [PubMed: 18571638]

Sakai I, Sharief FS, Li SS. Molecular cloning and nucleotide sequence of the cDNA for sperm-specific lactate dehydrogenase-C from mouse. Biochem. J. 1987; 242:619–622. [PubMed: 2439071]

Santi CM, Martínez-López P, de la Vega-Beltrán JL, Butler A, Alisio A, Darszon A, Salkoff L. The SLO3 sperm-specific potassium channel plays a vital role in male fertility. FEBS Letters. 2010; 584:1041–1046. [PubMed: 20138882]

Schreiber M, Wei A, Yuan A, Gaut J, Saito M, Salkoff L. Slo3, a novel pH-sensitive K+ channel from mammalian spermatocytes. J. Biol. Chem. 1998; 273:3509–3516. [PubMed: 9452476]

Seligman J, Zipser Y, Kosower NS. Tyrosine phosphorylation, thiol status, and protein tyrosine phosphatase in rat epididymal spermatozoa. Biol. Reprod. 2004; 71:1009–1015. [PubMed: 15151929]

Shum WWC, Da Silva N, Brown D, Breton S. Regulation of luminal acidification in the male reproductive tract via cell-cell crosstalk. J. Exp. Biol. 2009; 212:1753–1761. [PubMed: 19448084]

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; 25:605–617. [PubMed: 15223849]

Spungin B, Breitbart H. Calcium mobilization and influx during sperm exocytosis. J. Cell. Sci. 1996; 109(Pt 7):1947–1955. [PubMed: 8832417]

Spungin B, Margalit I, Breitbart H. Sperm exocytosis reconstructed in a cell-free system: evidence for the involvement of phospholipase C and actin filaments in membrane fusion. J. Cell. Sci. 1995; 108(Pt 6):2525–2535. [PubMed: 7673366]

Stock CE, Fraser LR. The acrosome reaction in human sperm from men of proven fertility. Hum. Reprod. 1987; 2:109–119. [PubMed: 3584413]

Storey BT, Kayne FJ. Energy metabolism of spermatozoa. V. The Embden-Myerhof pathway of glycolysis: activities of pathway enzymes in hypotonically treated rabbit epididymal spermatozoa. Fertil. Steril. 1975; 26:1257–1265. [PubMed: 803042]

Storey BT. Mammalian sperm metabolism: oxygen and sugar, friend and foe. Int. J. Dev. Biol. 2008; 52:427–437. [PubMed: 18649255]

Strunker T, Goodwin N, Brenker C, Kashikar ND, Weyand I, Seifert R, Kaupp UB. The CatSper channel mediates progesterone-induced Ca2+ influx in human sperm. Nature. 2011; 471:382–386. [PubMed: 21412338]

Suarez SS, Vincenti L, Ceglia MW. Hyperactivated motility induced in mouse sperm by calcium ionophore A23187 is reversible. J. Exp. Zool. 1987; 244:331–336. [PubMed: 3123593]

Suarez SS. Control of hyperactivation in sperm. Hum. Reprod. Update. 2008; 14:647–657. [PubMed: 18653675]

Sullivan R, Frenette G, Girouard J. Epididymosomes are involved in the acquisition of new sperm proteins during epididymal transit. Asian J. Androl. 2007; 9:483–491. [PubMed: 17589785]

Syntin P, Dacheux F, Druart X, Gatti JL, Okamura N, Dacheux JL. Characterization and identification of proteins secreted in the various regions of the adult boar epididymis. Biol. Reprod. 1996; 55:956–974. [PubMed: 8902205]

Tsai P-S, Garcia-Gil N, van Haeften T, Gadella BM. How Pig Sperm Prepares to Fertilize: Stable Acrosome Docking to the Plasma Membrane. PLoS ONE. 2010; 5:e11204. [PubMed: 20585455]

Vijayaraghavan S, Liberty GA, Mohan J, Winfrey VP, Olson GE, Carr DW. Isolation and molecular characterization of AKAP110, a novel, sperm-specific protein kinase A-anchoring protein. Mol. Endocrinol. 1999; 13:705–717. [PubMed: 10319321]

Vijayaraghavan S, Stephens DT, Trautman K, Smith GD, Khatra B, da Cruz e Silva EF, Greengard P. Sperm motility development in the epididymis is associated with decreased glycogen synthase kinase-3 and protein phosphatase 1 activity. Biol. Reprod. 1996; 54:709–718. [PubMed: 8835395]

Visconti PE, Krapf D, de la Vega-Beltran JL, Acevedo JJ, Darszon A. Ion channels, phosphorylation and mammalian sperm capacitation. Asian J Androl. 2011; 13:395–405. [PubMed: 21540868]

Wang D, Hu J, Bobulescu IA, Quill TA, McLeroy P, Moe OW, Garbers DL. A sperm-specific Na+/H+ exchanger (sNHE) is critical for expression and in vivo bicarbonate regulation of the soluble adenylyl cyclase (sAC). Proc. Natl. Acad. Sci. U.S.A. 2007; 104:9325–9330. [PubMed: 17517652]

Wang D, King SM, Quill TA, Doolittle LK, Garbers DL. A new sperm-specific Na+/H+ exchanger required for sperm motility and fertility. Nat. Cell Biol. 2003; 5:1117–1122. [PubMed: 14634667]

Welch JE, Schatte EC, O'Brien DA, Eddy EM. Expression of a glyceraldehyde 3-phosphate dehydrogenase gene specific to mouse spermatogenic cells. Biol. Reprod. 1992; 46:869–878. [PubMed: 1375514]

Wertheimer EV, Salicioni AM, Liu W, Trevino CL, Chavez J, Hernández-González EO, Darszon A, Visconti PE. Chloride is essential for capacitation and for the capacitation-associated increase in tyrosine phosphorylation. J. Biol. Chem. 2008; 283:35539–35550. [PubMed: 18957426]

Wissenbach U, Schroth G, Philipp S, Flockerzi V. Structure and mRNA expression of a bovine trp homologue related to mammalian trp2 transcripts. FEBS Lett. 1998; 429:61–66. [PubMed: 9657384]

Wood CD, Darszon A, Whitaker M. Speract induces calcium oscillations in the sperm tail. The Journal of Cell Biology. 2003; 161:89–101. [PubMed: 12695500]

Xia J, Reigada D, Mitchell CH, Ren D. CATSPER channel-mediated Ca2+ entry into mouse sperm triggers a tail-to-head propagation. Biol. Reprod. 2007; 77:551–559. [PubMed: 17554080]

Xia J, Ren D. Egg coat proteins activate calcium entry into mouse sperm via CATSPER channels. Biol. Reprod. 2009; 80:1092–1098. [PubMed: 19211808]

Xie F, Garcia MA, Carlson AE, Schuh SM, Babcock DF, Jaiswal BS, Gossen JA, Esposito G, van Duin M, Conti M. Soluble adenylyl cyclase (sAC) is indispensable for sperm function and fertilization. Dev. Biol. 2006; 296:353–362. [PubMed: 16842770]

Xu WM, Shi QX, Chen WY, Zhou CX, Ni Y, Rowlands DK, Yi Liu G, Zhu H, Ma ZG, Wang XF, Chen ZH, Zhou SC, Dong HS, Zhang XH, Chung YW, Yuan YY, Yang WX, Chan HC. Cystic fibrosis transmembrane conductance regulator is vital to sperm fertilizing capacity and male fertility. Proc. Natl. Acad. Sci. U.S.A. 2007; 104:9816–9821. [PubMed: 17519339]

Yudin AI, Gottlieb W, Meizel S. Ultrastructural studies of the early events of the human sperm acrosome reaction as initiated by human follicular fluid. Gamete Res. 1988; 20:11–24. [PubMed: 3235025]

Zanetti N, Mayorga LS. Acrosomal swelling and membrane docking are required for hybrid vesicle formation during the human sperm acrosome reaction. Biol. Reprod. 2009; 81:396–405. [PubMed: 19369646]

Zeng X-H, Yang C, Kim ST, Lingle CJ, Xia X-M. Deletion of the Slo3 gene abolishes alkalization-activated K+ current in mouse spermatozoa. Proceedings of the National Academy of Sciences. 2011; 108:5879–5884.

Zhang X, Zeng X, Xia X-M, Lingle CJ. pH-regulated Slo3 K+ Channels: Properties of Unitary Currents. The Journal of General Physiology. 2006; 128:301–315. [PubMed: 16940554]

Zippin JH, Levin LR, Buck J. CO(2)/HCO(3)(−)-responsive soluble adenylyl cyclase as a putative metabolic sensor. Trends Endocrinol. Metab. 2001; 12:366–370. [PubMed: 11551811]

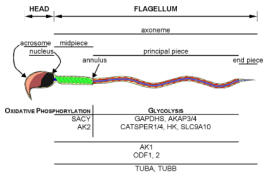

Figure 1.

Schematic drawing of a mouse sperm, indicating the physical compartments (head and flagellum) and their respective sub-compartments. Some of the functions or activities discussed in this review are diagrammatically represented below the drawing of the sperm. Guide to structures: acrosome (pink), nucleus (black), mitochondria (green), axoneme (blue), fibrous sheath (red), outer dense fibers (lavender), and cytoplasm (yellow). Also depicted are the compartments within the sperm head (acrosome, nucleus) and flagellum (axoneme, midpiece, principal piece, end piece). Mitochondria in the midpiece compartmentalize oxidative phosphorylation whereas glycolysis is restricted to the fibrous sheath. Examples of proteins localized within in the flagellum are: AK1 (adenylate kinase 1), AK2 (adenylate kinase 2), AKAP 3/4 (A-kinase anchoring protein 3/4), CATSPER 1/4 (cation channel, sperm associated 1/4), GAPDHS (glyceraldehyde phosphate dehydrogenase, spermatogenic), HK1-sc (hexokinase 1, spermatic cell), ODF 1/2 (outer dense protein 1/2), SACY (soluble adenylyl cyclase), SLC9A10 (solute carrier family 9, member 10; sNHE; sperm-specific Na+/H+ exchanger), TUBA (tubulin, alpha), TUBB (tubulin, beta).

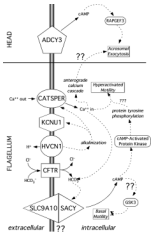

Figure 2.

Diagram of the activation pathways operative in sperm. This flowchart assimilates many of the pathways described for sperm from mouse and human, and presents the pathways using the mouse nomenclature. The diagram is by no means complete, and not all paths may eventually be shown to be accurate for the mouse. It is presented to serve as a conceptual framework. Question marks (??) indicate three areas that need further experimental investigational support: the role of an anterograde calcium cascade from the tail to the head, the intermediate steps between cAMP and GSK3, and the interaction between SLC9A10 (solute carrier family 9, member 10; sNHE; sperm-specific Na+/H+ exchanger) and SACY (soluble adenylyl cyclase). Other proteins depicted include: ADCY3 (adenylate cyclase 3), CATSPER (cation channel, sperm associated), CFTR (cystic fibrosis transmembrane conductance regulator), GSK3 (glycogen synthase kinase 3), HVCN1 (hydrogen voltage-gated channel; homolog of human Hv1), KCNU1 (potassium channel, subfamily U, member 1; SLO3), RAPGEF3 (Rap guanine nucleotide exchange factor 3; EPAC). For the sake of clarity, not all of the steps in sperm activation are presented.

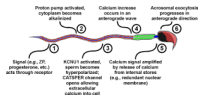

Figure 3.

Speculative model of how the flagellum could serve as a sensory organelle of mammalian sperm and lead to a physiological response such as acrosomal exocytosis. This model assumes that the sperm has undergone all necessary steps of maturation to be competent to respond to an external signal. 1) An extracellular ligand binds to and activates a receptor compartmentalized in the principal piece of the sperm flagellum. 2) As a result of receptor activation, the plasma membrane proton pump becomes activated and the cytoplasm becomes alkalinized. 3) As a result of the intracellular pH increase, KCNU1 is activated, leading to the hyperpolarization of the membrane, and CATSPER channels open, allowing extracellular calcium to enter the sperm. 3) Internal calcium increases throughout principal piece of the sperm, causing a wave that progresses in an anterograde direction toward the sperm head. 4) As the calcium wave progresses, the signal is amplified by the release of calcium from internal stores, such as the redundant nuclear envelope near the head-tail junction. 5) In response to the wave of calcium emanating forward from the flagellum, acrosomal exocytosis progresses in an anterograde direction.