Ca\(^{2+}\) -stores in sperm: their identities and functions

Sarah Costello\(^1,3\), Francesco Michelangeli\(^1\), Katherine Nash\(^1,3\), Linda Lefievre\(^1,3\), Jennifer Morris\(^1,3\), Gisela Machado-Oliveira\(^4\), Christopher Barratt\(^5\), Jackson Kirkman-Brown\(^2,3\) and Stephen Publicover\(^1,3\)

\(^1\)School of Biosciences, University of Birmingham, Birmingham B15 2TT, UK, \(^2\)Reproductive Biology and Genetics Research Group, The Medical School, University of Birmingham, Birmingham B15 2TT, UK, \(^3\)Centre for Human Reproductive Science, Birmingham Women’s Hospital, Birmingham B15 2TG, UK, \(^4\)School of Health Sciences, Piaget Institute, Algarve 6300-025, Silves, Portugal and \(^5\)Division of Maternal and Child Health Sciences, Medical School, Ninewells Hospital, University of Dundee, Dundee DD1 9SY, UK

Correspondence should be addressed to S Publicover at School of Biosciences, University of Birmingham, B15 2TT; Email: s.j.publicover@bham.ac.uk

Abstract

Intracellular Ca\(^{2+}\) stores play a central role in the regulation of cellular [Ca\(^{2+}\)]\(_i\) and the generation of complex [Ca\(^{2+}\)] signals such as oscillations and waves. Ca\(^{2+}\) signalling is of particular significance in sperm cells, where it is a central regulator in many key activities (including capacitation, hyperactivation, chemotaxis and acrosome reaction) yet mature sperm lack endoplasmic reticulum and several other organelles that serve as Ca\(^{2+}\) stores in somatic cells. Here, we review i) the evidence for the expression in sperm of the molecular components (pumps and channels) which are functionally significant in the activity of Ca\(^{2+}\) stores of somatic cells and ii) the evidence for the existence of functional Ca\(^{2+}\) stores in sperm. This evidence supports the existence of at least two storage organelles in mammalian sperm, one in the acrosomal region and another in the region of the sperm neck and midpiece. We then go on to discuss the probable identity of these organelles and their discrete functions: regulation by the acrosome of its own secretion and regulation by membranous organelles at the sperm neck (and possibly by the mitochondria) of flagellar activity and hyperactivation. Finally, we consider the ability of the sperm discretely to control mobilisation of these stores and the functional interaction of stored Ca\(^{2+}\) at the sperm neck/midpiece with CatSper channels in the principal piece in regulation of the activities of mammalian sperm.


[Ca\(^{2+}\)]\(_i\): a central regulator in sperm function

Regulation of cellular activity, in response to signals from other cells or from the extracellular environment, can occur at a number of levels. Long-term regulation is achieved by control of gene expression. This process can occur through control of translation and/or transcription and also by more subtle regulation of mRNA transcripts, and by regulation of turnover of the protein product. Effects of this type are typically exerted over time periods measured in hours rather than minutes or seconds. Regulation of cellular activity over shorter time periods is achieved by rapid, ‘post-translational’ modification of the function of proteins already present. Various pathways have been characterised, by which the actions of extracellular signals such as hormones, growth factors and transmitters are transduced, leading to appropriate modification of protein function. One such mechanism is through changes in the intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)).

In sperm, which lack endoplasmic reticulum (ER) and have a highly condensed nucleus, the regulation of function by translation/transcription (if it occurs at all) will be very limited. Post-translational mechanisms must, therefore, control all activities of the cell. Regulation of protein function through Ca\(^{2+}\) signalling is central to a range of activities that are pivotal to sperm function, including hyperactivation, chemotaxis and acrosome reaction (Publicover et al. 2007). Impairment of Ca\(^{2+}\) signalling in sperm is associated with male subfertility (Krausz et al. 1995, Baldi et al. 1999, Espino et al. 2009).

Signalling though [Ca\(^{2+}\)]\(_i\) is achieved by permitting Ca\(^{2+}\) to enter the cytoplasm (where concentration is maintained very low) from the extracellular space and/or from intracellular organelles, where the Ca\(^{2+}\) concentration is up to four orders of magnitude higher. Signal initiation requires merely that Ca\(^{2+}\) permeable membrane channels are opened, allowing the ions to flow down their electrochemical gradient. The presence of Ca\(^{2+}\) channels in the plasma membrane of sperm cells is well established, as is their significance in the key activities of sperm. A number of thorough reviews
on the various types and distribution of these channels are available (Darszon et al. 1999, Felix 2005, Jimenez-Gonzalez et al. 2006, Navarro et al. 2008). Here, we will review evidence for the existence, identity and role(s) of Ca\(^{2+}\) storage organelles in sperm.

### Ca\(^{2+}\) stores in somatic cells and their associated Ca\(^{2+}\) transporters

Somatic cells contain a number of membrane-bound organelles that undertake various biochemical reactions vital to the maintenance of cellular homeostasis and viability (Berridge et al. 1998). Many of these organelles also act as Ca\(^{2+}\) reservoirs or Ca\(^{2+}\) stores which contribute to the regulation of Ca\(^{2+}\)-dependent processes (Michelangeli et al. 2005). In order to be classified as a \textit{bona fide} Ca\(^{2+}\) store, an organelle must have at least two types of Ca\(^{2+}\) transporters, enabling both loading of the store and release of stored Ca\(^{2+}\) in a controlled fashion.

### Ca\(^{2+}\) uptake and release mechanisms

Ca\(^{2+}\) accumulation into stores normally occurs against the electrochemical gradient for the ion and therefore requires expenditure of energy. Typically this is achieved by ATPase ‘pumps’ such as the sarcoplasmic–ER Ca\(^{2+}\) ATPase (SERCA) or secretory pathway Ca\(^{2+}\) ATPases (SPCA), though Ca\(^{2+}\) exchangers (co-transporters) may also be used. By contrast, controlled release of Ca\(^{2+}\) can be achieved by gating of Ca\(^{2+}\)-permeable ion channels in the membrane of the organelle. These are usually regulated by second messengers (or putative second messengers) such as inositol 1,4,5-trisphosphate (IP\(_3\)), cyclic ADP ribose (cADP-ribose), nicotinic acid ADP (NAADP) and even by Ca\(^{2+}\) itself, via a Ca\(^{2+}\)-induced Ca\(^{2+}\) release mechanism (CICR; Bootman et al. 2001). The difference in mechanisms for uptake and release of stored Ca\(^{2+}\) has significant effects upon the rates at which Ca\(^{2+}\) translocation occurs. SERCAs must undergo multiple binding and conformational states during translocation of Ca\(^{2+}\) and transport only a few ions per ATPase molecule per second, whereas a single release channel can transport 100,000’s of Ca\(^{2+}\) in the same period (Taylor 1995).

### Ca\(^{2+}\) storage organelles

**Endoplasmic reticulum**

In the early 1980s it was first demonstrated that the ER acted as a Ca\(^{2+}\) store that could release its Ca\(^{2+}\) in the presence of the agonist-generated second messenger IP\(_3\) (Berridge 2002). This release was later shown to occur via activation of IP\(_3\) receptors (IP\(_3\)Rs), IP\(_3\)-activated Ca\(^{2+}\) channels located on the ER membranes (Michelangeli et al. 1995). From analogous studies on striated muscle sarcoplasmic reticulum (SR), it was shown that ER membranes also contain SERCA pumps for Ca\(^{2+}\) accumulation and ryanodine receptor (RyR) type Ca\(^{2+}\) channels, named for their sensitivity to the drug ryanodine, but activated \textit{in vivo} by Ca\(^{2+}\) itself (CICR) and possibly by cADP-ribose (Michelangeli et al. 2005). Though there seems little doubt that the ER is the primary store of Ca\(^{2+}\) that is used in intracellular Ca\(^{2+}\) signalling, other organelles may also play a role (Michelangeli et al. 2005).

**Nuclear, golgi and lysosomal Ca\(^{2+}\) storage**

Immunohistochemical and biochemical studies have shown that the nuclear envelope, the outer membrane of which is continuous with the ER, also contains both SERCA Ca\(^{2+}\) pumps and IP\(_3\)R Ca\(^{2+}\) channels (Lanini et al. 1992, Humbert et al. 1996). RyR type Ca\(^{2+}\) channels have also been identified on the nuclear membrane (Gerasimenko et al. 2003). In some cells the nuclear membrane forms a complex tubular network which penetrates deep into the nucleus and which is particularly enriched in IP\(_3\)Rs (Echevarria et al. 2003). This has lead to the suggestion that Ca\(^{2+}\) mobilisation, leading to localised increases in [Ca\(^{2+}\)] within distinct regions of the nucleus, may affect gene transcription.

The Golgi apparatus, involved in both post-translational protein modification and protein trafficking, has also been shown to contain IP\(_3\)Rs and SERCA Ca\(^{2+}\) pumps (Surroca & Wolff 2000). These transporters are localised to the \textit{cis} Golgi region, while membranes of the \textit{trans} Golgi region contain the SPCA pump (Missiaen et al. 2004, Wootton et al. 2004), which has different transport properties compared to SERCA.

A role for lysosomes in Ca\(^{2+}\) signalling is suggested by the observation that they release Ca\(^{2+}\) when treated with the NAD metabolite and putative second messenger NAADP, which activates the NAADP-sensitive Ca\(^{2+}\) channel (Churchill et al. 2002, Kinneer et al. 2004) of the two-pore channel family (Calcraft et al. 2009). These organelles are believed to be filled by a H\(^+\)/Ca\(^{2+}\) exchanger utilising the proton gradient across the membrane maintained by the vacuolar H\(^+\) ATPase (Churchill et al. 2002).

**Mitochondria**

It has been known for some time that mitochondria can accumulate Ca\(^{2+}\) into the matrix space, primarily through the mitochondrial Ca\(^{2+}\) uniporter (MCU) located on the inner mitochondrial membrane. Ca\(^{2+}\) uptake is driven by the negative membrane potential of the mitochondrial matrix. Recent studies have shown the MCU to be a Ca\(^{2+}\) channel of relatively low conductance, with a complex gating mechanism (Kirichok et al. 2004). Controlled release of mitochondrial Ca\(^{2+}\) can occur through a Na\(^+\)/Ca\(^{2+}\) exchanger (Bernardi 1999). Under conditions where ‘resting’ [Ca\(^{2+}\)]\(_{i}\) is
elevated, Ca\textsuperscript{2+} uptake by mitochondria both activates a number of key tricarboxylic acid cycle dehydrogenases and also acts as a Ca\textsuperscript{2+} sink in order to buffer cytosolic Ca\textsuperscript{2+} levels (Gunter et al. 2004). If excessive mitochondrial Ca\textsuperscript{2+} accumulation occurs this can lead to activation of the permeability transition pore, which permits release from the mitochondrial matrix of factors that initiate cell death (Orrenius et al. 2003, Dong et al. 2006, Jeong & Seol 2008). However, under physiological conditions mitochondria also play an important role in Ca\textsuperscript{2+} buffering and signalling, shaping (and often extending) the kinetics of Ca\textsuperscript{2+} signals (Bianchi et al. 2004, Rimessi et al. 2008).

Over the last few years researchers have begun to investigate potential interactions between different Ca\textsuperscript{2+} stores, some recent evidence indicating that such interactions may contribute to the complexity of spatio-temporal intracellular [Ca\textsuperscript{2+}] profiles (Michelangeli et al. 2005). Current research is now focussing on identifying these interactions and assessing their roles in controlling complex physiological processes.

Do sperm have Ca\textsuperscript{2+} stores?

In somatic cells the ER is the primary Ca\textsuperscript{2+} storage organelle. A mature sperm has no recognisable ER but does have a nuclear membrane, an acrosome (a single cap-shaped vesicle that surrounds the anterior nucleus), mitochondria (which are concentrated in the midpiece) and some poorly-defined, irregular membranous structures in the region of the sperm neck from where the cytoplasmic droplet has been shed (Fig. 1a). Since organelles other than the ER can participate in storage and release of Ca\textsuperscript{2+} in somatic cells (see section Ca\textsuperscript{2+} storage organelles above), any or several of the membranous structures of sperm may act as releasable Ca\textsuperscript{2+} stores.

Components of Ca\textsuperscript{2+} storage organelles expressed in sperm

\textbf{IP\textsubscript{3} receptors}

The first clear evidence that intracellular organelles in mature mammalian sperm might act as Ca\textsuperscript{2+} stores was the finding of Walensky & Snyder (1995) that components of the phosphoinositide signalling system are present in mammalian sperm. Both the G protein G\textsubscript{a}\textsubscript{q/11} and the \(i\) isoform of phospholipase C (PLC), which generates the Ca\textsuperscript{2+}-mobilising intracellular ligand \(i\), were identified in the acrosomal (anterior head) region. \(i\)Rs were also present, primarily in the anterior head, though a second, smaller concentration of receptors was detected in the anterior midpiece. \(i\)Rs were enriched in acrosomal fractions and were lost from the sperm into the medium upon acrosome reaction, consistent with localisation to the outer acrosomal membrane.

\textbf{Tomes et al. (1996)} identified PLC\(\gamma\) in the head of mouse sperm. The enzyme was transferred to the particulate fraction during capacitation. Stimulation of the sperm with solubilised zona pellucida increased tyrophostin-sensitive PLC activity, suggesting that the \(\gamma\) isoform of PLC was activated during induction of acrosome reaction. Since these initial reports, the presence of \(i\)Rs in the sperm of a number of mammals has been confirmed (Dragileva et al. 1999, Kuroda et al. 1999,
SERCAs are present in sperm; their role is far from clear
sea urchin (Gunaratne & Vacquier 2006; Fig. 1f and g). If
and e). Similar results were obtained with sperm of the
varied somewhat between species and between studies,
Though the exact pattern of staining that was reported
sperm plasma membrane (Zapata
anterior midpiece (Fig. 1b). In sea urchin sperm an IP 3-
over the acrosome and the other at the sperm neck or
Ca2+
channels so it is possible that RyRs, if present in sperm,
Ca2+
mobilisation of stored Ca2+ (Treiman
thapsigargin, a highly potent and specific blocker of
Ca2+
uptake pump and a cAMP-
A characteristic of Ca2+ stores in somatic cells is the
protein calreticulin, which acts as a chelator of Ca2+ within the storage organelle. This protein is present
in the acrosome of developing rat sperm (Nakamura
and neck regions of human and bovine sperm
Evidence for functional calcium storage in sperm
Direct assessment of uptake and release of Ca2+ by
sperm organelles or organelle membranes has been
attempted in only a few studies. Walensky & Snyder
(1995) measured accumulation and release of 45Ca2+
in digitonin-permeabilised rat sperm and demonstrated an
ATP-dependent accumulation of Ca2+ into an intra-
cellular site that was sensitive to thapsigargin (10 µM).
Accumulated Ca2+ was released (partially) by 10 µM IP3. Spungin & Breitbart (1996) reported that purified
acrosomal membranes from bovine sperm possessed a
thapsigargin-sensitive Ca2+ uptake pump and a CAM-
activated Ca2+-release channel. These authors
suggested that generation of cAMP (and consequent
mobilisation of stored Ca2+) could occur upon
interaction with the zona pellucida (Breitbart & Spungin
An alternative approach has been indirectly to assess Ca2+ movements attributable to store uptake and release in intact sperm by using fluorescent Ca2+ indicators to
monitor cytoplasmic [Ca2+]. Blackmore (1993) showed
that treatment of human sperm with the SERCA inhibitor
thapsigargin, to release Ca2+ from intracellular stores,
caus ed a sustained increase in [Ca2+]i due to opening of
channels at the plasma membrane. No elevation of
[Ca2+]i was seen when extracellular [Ca2+] was buffered
with EGTA but upon subsequent addition of Ca2+
to the extracellular medium there was a sustained
rise in [Ca2+]i. Rossato et al. (2001) and Williams & Ford
(2003) reported similar observations but in these studies
a transient (and much smaller) increase in [Ca2+]i
was also observed when the drug was applied to cells bathed in
Ca2+-free saline, confirming that mobilisation of
stored Ca2+ was indeed occurring. Similar types of
response have been observed in sperm of rams
(Dragileva et al. 1999), mice (O’Toole et al. 2000) and
sea urchins (Gonzalez-Martinez et al. 2004). The
simplest interpretation of these observations would be
that sperm possess an intracellular store (or stores) of
Ca2+ that can be mobilised by treatment with thapsi-
gargin. Mobilised Ca2+ may sometimes be insufficient
to cause a detectable elevation of [Ca2+]i, but nevertheless
can induce Ca2+ influx through store-operated (capaci-
tive) Ca2+ channels (see section Store-operated Ca2+
channels in sperm below).
The mechanism by which thapsigargin mobilises
stored Ca2+ in sperm is not clear. Rossato et al. (2001)
reported effects of the drug on Ca2+ handling by human

Ryanodine receptors
The evidence regarding expression of RyRs in mamma-
lian sperm is less clear. We have observed staining of
mature human sperm in the region of the sperm neck
both with BODIPY FL-X ryanodine (a fluorescently-
tagged ryanodine derivative) and with antibodies against
RyRs 1 and 2 (Harper et al. 2004, Lefievre et al. 2007). By
contrast, others have reported no staining with BODIPY
FL-X ryanodine in bovine sperm (Ho & Suarez 2001) and
staining only for RyR3 in mature rodent sperm (Treviso
et al. 1998). The conductance of RyRs (>100 pS; Zalk
et al. 2007) is particularly high for Ca2+-permeable
channels so it is possible that RyRs, if present in sperm,
are expressed at extremely low levels. Only one or two
channels may be present in each cell, Ca2+ flux
being regulated by the proportion of time for which the
channel is open as it flickers between open and closed states.

Ca2+ store pumps and Ca2+ chelating proteins
Rossato et al. (2001) used the BODIPY derivative of
thapsigargin, a highly potent and specific blocker of
SERCAs (Treiman et al. 1998), to probe for the presence of
SERCAs in human sperm. Similarly to staining patterns
for IP3Rs, localisation of BODIPY thapsigargin was
observed over the acrosome and also the midpiece.
More recently, Lawson et al. (2007), using antibodies to
SERCA type 2, obtained a similar pattern of staining in
human, bovine and mouse sperm. SERCA2 was also
detected by western blot. Subcellular fractionation
showed that binding occurred primarily in the mem-
brane fraction of the cells and also suggested that
different splice variants of SERCA2 were present in
different subcellular locations. By contrast, using immu-
nolocalisation and western blotting, we were unable to
detect SERCAs in human sperm using a pan-SERCA
antibody, but did detect SPCA1, another intracellular
Ca2+ pump. SPCA1 immunostaining was observed only
at the sperm neck/midpiece (Harper et al. 2005; Fig. 1d
and e). Similar results were obtained with sperm of the
sea urchin (Gunaratne & Vacquier 2006; Fig. 1f and g). If
SERCAs are present in sperm their role is far from clear
since mobilisation of stored Ca2+ in sperm by exposure
to thapsigargin requires high (non-specific) doses of the
drug (Harper et al. 2005; see section Evidence for
functional calcium storage in sperm below).

www.reproduction-online.org
Downloaded from Bioscientifica.com at 10/14/2018 01:05:39AM
via free access
sperm at 10–100 nM and Meizel & Turner (1993) observed dose-dependent induction of acrosome reaction at similar doses. These observations are consistent with studies on somatic cells where 50% block of SERCA activity (or 50% maximal Ca\textsuperscript{2+}-store mobilisation) occurs at <100 nM and often <10 nM thapsigargin (Treiman et al. 1998, Wootton & Michelangeli 2006). However, most studies on the effects of thapsigargin on sperm Ca\textsuperscript{2+} signalling have used micromolar doses (1–20 μM), with negligible effects being observed at doses ≤5 μM (e.g. Dragileva et al. 1999, Williams & Ford 2003, Harper et al. 2005). Cyclopiazonic acid (CPA), another widely used SERCA inhibitor, mobilised Ca\textsuperscript{2+} in human sperm at high doses (maximal effect at 100 μM; Rossato et al. 2001) but completely fails to mobilise Ca\textsuperscript{2+} at lower doses (Williams & Ford 2003, Harper et al. 2005) that could be considered both saturating and specific (Wootton & Michelangeli 2006). Thus, though it appears that SERCA (at least SERCA2) is expressed in mammalian sperm (see section Components of Ca\textsuperscript{2+} storage organelles expressed in sperm), such that mechanisms for Ca\textsuperscript{2+} stores in intact sperm may reflect non-specific actions at non-SERCA sites.

Work in our own laboratory has provided evidence for participation of stored Ca\textsuperscript{2+} in complex [Ca\textsuperscript{2+}], signals that occur in human sperm stimulated with progesterone or NO, both products of the female tract signalling have used micromolar doses (1–20 μM), with negligible effects being observed at doses ≤5 μM (e.g. Dragileva et al. 1999, Williams & Ford 2003, Harper et al. 2005). Cyclopiazonic acid (CPA), another widely used SERCA inhibitor, mobilised Ca\textsuperscript{2+} in human sperm at high doses (maximal effect at 100 μM; Rossato et al. 2001) but completely fails to mobilise Ca\textsuperscript{2+} at lower doses (Williams & Ford 2003, Harper et al. 2005) that could be considered both saturating and specific (Wootton & Michelangeli 2006). Thus, though it appears that SERCA (at least SERCA2) is expressed in mammalian sperm (see section Components of Ca\textsuperscript{2+} storage organelles expressed in sperm), such that mechanisms for Ca\textsuperscript{2+} stores in intact sperm may reflect non-specific actions at non-SERCA sites.

Work in our own laboratory has provided evidence for participation of stored Ca\textsuperscript{2+} in complex [Ca\textsuperscript{2+}], signals that occur in human sperm stimulated with progesterone or NO, both products of the female tract production of IP\textsubscript{3} but does not fluoresce significantly at the high Ca\textsuperscript{2+} concentrations inside the Ca\textsuperscript{2+} storage organelles but does not fluoresce significantly at the much lower Ca\textsuperscript{2+} concentration in the cytoplasm (Herrick et al. 2005, Morris J unpublished data; Fig. 2a). Intriguingly, immunolocalisation studies indicate that the ‘toolkits’ of these two stores may differ (section Components of Ca\textsuperscript{2+} storage organelles expressed in sperm), such that mechanisms for Ca\textsuperscript{2+} mobilisation and accumulation at the two sites within the cell may be discrete (section Separation of store-regulated activities below; Publicover et al. 2007).

The acrosomal store
There is no dispute that the storage organelle in the acrosomal region of the sperm head is the acrosome (Fig. 1a) itself. Ca\textsuperscript{2+} release channels (IP\textsubscript{3}Rs) in this region occur primarily (possibly exclusively) in the outer acrosomal membrane (see section Components of Ca\textsuperscript{2+} storage organelles expressed in sperm). Thus, the acrosomal store regulates Ca\textsuperscript{2+} concentration in the peri-acrosomal cytoplasm, its pumps and channels being lost during acrosome reaction when compound fusion occurs between the outer acrosomal membrane and the overlying plasmalemma.

Ca\textsuperscript{2+} storage at the sperm neck/midpiece
The identity of the Ca\textsuperscript{2+} storage organelle in the neck/midpiece region of the sperm is less clear. Suarez et al. have demonstrated the presence of IP\textsubscript{3}Rs and calreticulin (a Ca\textsuperscript{2+} binding and storage protein) at the neck region of bovine and hamster sperm, in the region occupied by the redundant nuclear envelope...
effect of NO on the Ca\textsuperscript{2+} mitochondrial membrane potential. When dithiothreitol (DTT) is sperm neck/midpiece by a mechanism that does not require an intact gap (arrows) between these stripes and the sperm neck exactly as the occurring as two ‘stripes’ of highly fluorescent points with a small was a concentration of fluorescence in the mitochondrial midpiece, elevation of [Ca\textsuperscript{2+}] oscillations reflect cyclical release and re-uptake of Ca\textsuperscript{2+} which sensitises the Ca\textsuperscript{2+} inner membrane potential, there is a pronounced rise in [Ca\textsuperscript{2+}]i (Machado-Oliveira et al. 2001) observed co-localisation of IP\textsubscript{3}Rs and calreticulin in both the acrosome and neck region of human sperm. Immunogold staining for calreticulin showed that this protein was present in the acrosome (particularly at the equatorial segment) and also in vesicles in the sperm neck (adjacent to the nucleus) and in the cytoplasmic droplet (Fig. 2c). These vesicles were closely apposed to the plasma membrane (Naaby-Hansen et al. 2001).

A second candidate for intracellular storage of Ca\textsuperscript{2+} in the neck/midpiece region of sperm is accumulation and release by mitochondria (see section Mitochondria). Mitochondria of mammalian sperm have been shown to take up Ca\textsuperscript{2+} in situ (Storey & Keyhani 1973, 1974, Babcock et al. 1976, Vijayaraghavan & Hoskins 1990). In mouse sperm the contribution of mitochondrial Ca\textsuperscript{2+} buffering was marginal under resting conditions but became more significant when plasma membrane Ca\textsuperscript{2+} pumps were inhibited, conditions under which resting [Ca\textsuperscript{2+}]i may be elevated (Wennemuth et al. 2003). Occasionally we have observed strong fluorescence, apparently localized to the mitochondria, in human sperm labelled with Ca\textsuperscript{2+}-reporting dyes (Fig. 2d and e), suggesting that these organelles were accumulating large amounts of Ca\textsuperscript{2+}. This was particularly characteristic of one donor who was known to be fertile and did not appear to be associated with reduced cell viability or function.

Conventional mitochondrial Ca\textsuperscript{2+} uptake and release does not contribute significantly to the store-mediated Ca\textsuperscript{2+}-oscillations that occur in the posterior head and midpiece of human sperm stimulated with low doses of progesterone or with NO. Uncoupling of mitochondrial respiration (with 2,4-dinitrophenol or CCCP) does not inhibit these [Ca\textsuperscript{2+}]i oscillations and release despite the continued presence of the mitochondrial uncoupler. Panel b from Ho & Suarez (2003) with permission, panel c from Naaby-Hansen et al. (2001) with permission.

(RNE; Figs 1b and 2b) the ‘excess’ nuclear membrane that accumulates due to nuclear condensation and is packaged at the sperm neck. Since this membrane is continuous with the ER in the immature cell, it may even include vestiges of functional ER membrane. Staining for nuclear pore complex proteins (markers for the RNE) only partial overlapped that for IP\textsubscript{3}Rs (Ho & Suarez 2003). Immunogold labelling of electron microscope sections showed that calreticulin and IP\textsubscript{3}Rs were associated with membrane cisternae that did not contain nuclear pores and were apparently a separate compartment of the RNE (Ho & Suarez 2001, 2003). No staining was associated with mitochondria. Pharmacological manipulations designed to activated these receptors (e.g. thimerosal) or to inhibit intracellular Ca\textsuperscript{2+} pumps (5–20 \mu M thapsigargin) mobilised Ca\textsuperscript{2+} in the region of the sperm neck and had functional effects (see below) on motility (Ho & Suarez 2001, 2003). Naaby-Hansen et al. (2001) observed co-localisation of IP\textsubscript{3}Rs and calreticulin in both the acrosome and neck region of human sperm. Immunogold staining for calreticulin showed that this protein was present in the acrosome (particularly at the equatorial segment) and also in vesicles in the sperm neck (adjacent to the nucleus) and in the cytoplasmic droplet (Fig. 2c). These vesicles were closely apposed to the plasma membrane (Naaby-Hansen et al. 2001).

A second candidate for intracellular storage of Ca\textsuperscript{2+} in the neck/midpiece region of sperm is accumulation and release by mitochondria (see section Mitochondria). Mitochondria of mammalian sperm have been shown to take up Ca\textsuperscript{2+} in situ (Storey & Keyhani 1973, 1974, Babcock et al. 1976, Vijayaraghavan & Hoskins 1990). In mouse sperm the contribution of mitochondrial Ca\textsuperscript{2+} buffering was marginal under resting conditions but became more significant when plasma membrane Ca\textsuperscript{2+} pumps were inhibited, conditions under which resting [Ca\textsuperscript{2+}]i may be elevated (Wennemuth et al. 2003). Occasionally we have observed strong fluorescence, apparently localized to the mitochondria, in human sperm labelled with Ca\textsuperscript{2+}-reporting dyes (Fig. 2d and e), suggesting that these organelles were accumulating large amounts of Ca\textsuperscript{2+}. This was particularly characteristic of one donor who was known to be fertile and did not appear to be associated with reduced cell viability or function. Conventional mitochondrial Ca\textsuperscript{2+} uptake and release does not contribute significantly to the store-mediated Ca\textsuperscript{2+}-oscillations that occur in the posterior head and midpiece of human sperm stimulated with low doses of progesterone or with NO. Uncoupling of mitochondrial respiration (with 2,4-dinitrophenol or CCCP) does not inhibit these [Ca\textsuperscript{2+}]i oscillations and release despite the continued presence of the mitochondrial uncoupler. Panel b from Ho & Suarez (2003) with permission, panel c from Naaby-Hansen et al. (2001) with permission.
STIM1, a marker of Ca\(^{2+}\) stores (see section Store-operated Ca\(^{2+}\) channels in sperm below), also stains the length of the midpiece. Two distinct ‘stripes’ of staining are often discernible in STIM1 stained cells, consistent with localization to the mitochondria and similar to the pattern of staining seen when mitochondria are Ca\(^{2+}\)-loaded (Fig. 3). However, it should be noted that, in sperm of the sea urchin Stronglocentrotus purpuratus, mitochondrial inhibitors and uncouplers cause mobilization of stored Ca\(^{2+}\) followed by sustained Ca\(^{2+}\) influx, apparently due to mobilization of mitochondrial Ca\(^{2+}\) and consequent activation of store-operated Ca\(^{2+}\) channels (Ardón et al. 2009).

**Store-operated Ca\(^{2+}\) channels in sperm**

A common observation in somatic cells is that mobilisation of stored Ca\(^{2+}\) (leading to a fall in \([\text{Ca}^{2+}]_\text{i}\) inside the storage organelle) causes activation of Ca\(^{2+}\) influx though store-operated Ca\(^{2+}\) channels at the plasma membrane. This process is called ‘store-operated’ or ‘capacitative’ Ca\(^{2+}\) entry. It appears that store-operated influx encompasses ‘a family of Ca\(^{2+}\) -permeable channels, with different properties in different cells’ (Parekh & Putney 2005). Evidence in support of the occurrence of store operated Ca\(^{2+}\) influx in mammalian and sea urchin sperm has been reported by a number of laboratories, all of whom observed increased influx of Ca\(^{2+}\) into sperm in response to manoeuvres designed to mobilise stored Ca\(^{2+}\) (Blackmore 1993, Dragileva et al. 1999, O’Toole et al. 2000, Rossato et al. 2001, Hirohashi & Vaccquer 2003, Williams & Ford 2003, Ardón et al. 2009, Espino et al. 2009).

Some members of the canonical transient receptor potential (TRPC) channel superfamily have been suggested as candidate store-operated channels in somatic cells (Birnbaumer et al. 1996, Abramowicz & Birnbaumer 2009). In mouse sperm, several TRPC channels are expressed and have been shown to be localised over the anterior sperm head (Jungnickel et al. 2001, Castellano et al. 2003, Sutton et al. 2004, Stamboulian et al. 2005). Channels incorporating TRPC2 play a role in the zona pellucida-induced Ca\(^{2+}\) influx that leads to acrosome reaction (Jungnickel et al. 2001), though whether activation of these channels is a response to Ca\(^{2+}\) store depletion is still not clear (Florman et al. 2008; see section The acrosomal store). More recently, the proteins of the STIM and ORAI families have been proposed to play key roles in capacitative Ca\(^{2+}\) influx. STIM1 is the putative sensor for detection of Ca\(^{2+}\) store status and ORAI1 is thought to form the Ca\(^{2+}\)-permeable membrane channel (Strange et al. 2007, Wang et al. 2008). These proteins may also combine/interact with TRPCs to form and regulate store-operated channels (Abramowicz & Birnbaumer 2009, Kim et al. 2009). The role(s) of these channels in sperm are far from clear but there is evidence to suggest that they may be important in acrosome reaction in mammalian and echinoderm sperm (O’Toole et al. 2000, Gonzalez-Martinez et al. 2004; see section The acrosomal store). We have examined the expression in human sperm of STIM and ORAI (K Nash & L Lefievre, unpublished data). Both western blotting and immunolocalisation confirm the presence of these proteins and suggest that they are present primarily at the sperm neck and midpiece, though lower levels of expression over the acrosomal region may also occur (Fig. 3).

**Roles of Ca\(^{2+}\) stores in sperm**

Since at least two intracellular Ca\(^{2+}\) stores are present in sperm, in different locations and potentially with different mechanisms of filling and mobilisation (Publicover et al. 2007), it is likely that these organelles have different roles in the regulation of sperm function.

**The acrosomal store**

The acrosomal store is strongly implicated in regulation of exocytosis of the acrosomal vesicle itself (acrosome reaction). Mouse sperm stimulated with zona pellucida glycoprotein ZP3 show a large, transient influx of Ca\(^{2+}\) (Arnoult et al. 1999) that may reflect activation of a T-type voltage-operated Ca\(^{2+}\) channel, though the identity of this channel is not yet established (Florman et al. 2008). In parallel to this Ca\(^{2+}\) influx there is believed to be a G-protein dependent elevation of pH\(_i\) and also activation of PLC leading to generation of IP\(_3\) (Florman et al. 2008). Male mice that are null for PLC\(_\delta\) show reduced fertility associated with failure of the sperm to undergo acrosome reaction upon binding to the zona pellucida (Fukami et al. 2001). Further investigation of sperm from these animals showed that they were unable to generate a \([\text{Ca}^{2+}]_\text{i}\) signal in response to solubilised zona pellucida, whereas the cells could respond normally to 5 \(\mu\)M thapsigargin

---

**Figure 3** STIM1 is expressed at the sperm neck and midpiece. (a) Immunolocalisation of STIM1 in human sperm. (b) Shows immunofluorescence of STIM1 overlaid on a phase image of the same cells. The fluorescence appears as two ‘stripes’ (as is seen in Ca\(^{2+}\)-loaded mitochondria; Fig. 2d), with a brighter spot in the region of the RNE and calreticulin-containing vesicles (arrows) anterior to the mitochondria (arrow in c).
(Fukami et al. 2003). De Blas et al. (2002) used streptolysin-O treated (permeabilised) human sperm directly to observe the status of the acrosomal Ca\textsuperscript{2+} store (see section Location and identity of the Ca\textsuperscript{2+} storage organelle(s) in sperm above). Using this approach, they were able to show that mobilisation of acrosomal Ca\textsuperscript{2+} through IP\textsubscript{3}-sensitive channels was required for induction of acrosome reaction by the small GTPase Rab3A. Herrick et al. (2005) used labelling of Ca\textsuperscript{2+} stores in intact mouse sperm (see section Location and identity of the Ca\textsuperscript{2+} storage organelle(s) in sperm above) to show a clear association between mobilisation of acrosomal Ca\textsuperscript{2+} (by 20 \mu M thapsigargin) and acrosome reaction. In cells bathed in medium containing no added Ca\textsuperscript{2+} and supplemented with 5 mM EGTA thapsigargin was still effective in inducing acrosome reaction. They concluded that mobilisation of the acrosomal store can be sufficient to induce acrosome reaction, such that the acrosome can be viewed as a Ca\textsuperscript{2+}-storage organelle that is capable of regulating its own secretion (Herrick et al. 2005).

After the initial activation of signalling that occurs upon contact with the egg vestments, there is a sustained influx of Ca\textsuperscript{2+} that is apparently required for acrosome reaction, both in mouse and sea urchin sperm (O’Toole et al. 2000, Gonzalez-Martinez et al. 2004). In mouse sperm, the plasma membrane channels incorporating TRPC2 subunits are implicated in this process (Jungnickel et al. 2001), potentially being activated by a store operated mechanism (O’Toole et al. 2000), though other mechanisms of activation are also possible (Florman et al. 2008). A role for store operated Ca\textsuperscript{2+}-influx in induction of acrosome reaction is consistent with observations that treatment of mammalian sperm with thapsigargin (to mobilise stored Ca\textsuperscript{2+}) causes both elevation of [Ca\textsuperscript{2+}]\textsubscript{i}, and acrosome reaction (Blackmore 1993, Meisel & Turner 1993, Dragileva et al. 1999, Rossato et al. 2001, Williams & Ford 2003), both these effects being dependent upon influx of extracellular Ca\textsuperscript{2+}. Since store operated Ca\textsuperscript{2+} influx may involve a combination of TRPC and ORAI subunits (section Store-operated Ca\textsuperscript{2+} channels in sperm), ORAI may also participate in this process. Store-operated Ca\textsuperscript{2+} influx is also implicated in acrosome reaction in sea urchin spermatozoa stimulated with egg jelly (Gonzalez-Martinez et al. 2004).

Models for activation of SNARE (membrane fusion) proteins during acrosome reaction typically incorporate a two-stage Ca\textsuperscript{2+} signal, but mobilisation of stored Ca\textsuperscript{2+} is the final ‘trigger’ of acrosome reaction (e.g. Mayorga et al. 2007, Zarelli et al. 2009). Requirement for store-operated Ca\textsuperscript{2+} influx downstream of mobilisation of the acrosomal store is thus not absolutely established. However, it should be noted that in experimental situations mobilisation of stored Ca\textsuperscript{2+} might be exaggerated, generating a [Ca\textsuperscript{2+}]\textsubscript{i} signal that is larger and more effective than that occurring upon zona pellucida binding.

Recently, the model (described above) for activation of sperm Ca\textsuperscript{2+} signalling by zona pellucida has been challenged. Xia & Ren (2009) reported that, in epididymal mouse sperm, the only functional plasma membrane Ca\textsuperscript{2+} channels were formed by CatSper, a family of sperm-specific, plasma membrane ion channel subunits. CatSper are localised to the principal piece of the flagellum, where they form weak voltage-sensitive, Ca\textsuperscript{2+}-permeable channels that are activated by elevated pH, and mediate hyperactivation (Navarro et al. 2008). Solubilised zona pellucida induced a [Ca\textsuperscript{2+}]\textsubscript{i} elevation in 66% of sperm that initiated (within 20 s of stimulation) at the principal piece and then spread forward, taking almost 3 s to reach the sperm head (Xia & Ren 2009). In 37% of cells, a second (delayed) response occurred a few minutes after stimulation. Zona pellucida could not induce the first elevation of [Ca\textsuperscript{2+}]\textsubscript{i} in any CatSper null sperm, but the delayed response occurred in 18% of these cells. Since zona pellucida receptors are likely to be in the sperm head activation of CatSper in the principal piece of the flagellum is probably indirect, possibly via zona pellucida-induced elevation of pH. CatsSper null sperm were able to undergo acrosome reaction in response to stimulation with zona pellucida, leading the authors to speculate that it was the delayed phase of the Ca\textsuperscript{2+} signal (possibly Ca\textsuperscript{2+} store generated) that induced acrosome reaction. The spread of elevated [Ca\textsuperscript{2+}]\textsubscript{i} from the principal piece into the head of wild-type cells is probably an active process (Xia & Ren 2009) and may well reflect mobilisation of Ca\textsuperscript{2+} stores by CICR. However, this model cannot easily be reconciled with the key role of voltage operated Ca\textsuperscript{2+} channels in the established model described above, for which there is a considerable body of evidence. Clearly, there is a need for further work in this area.

**Effects of mobilisation of Ca\textsuperscript{2+} stored at the neck/midpiece**

The store located in the region of the sperm neck functions as a regulator of sperm motility. Ho & Suarez showed that manoeuvres designed to mobilise this store (application of thapsigargin or the IP\textsubscript{3}R agonist thimerosal) caused elevation of [Ca\textsuperscript{2+}]\textsubscript{i} in the neck region and hyperactivation in bovine sperm, these effects being independent of [Ca\textsuperscript{2+}]\textsubscript{j} (Ho & Suarez 2001, 2003). Assessment of mitochondrial function and pharmacological manipulation of the mitochondrial Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger indicated that the observed effects did not reflect activity of conventional mitochondrial Ca\textsuperscript{2+} uptake and release mechanisms (Ho & Suarez 2003). They went on to show a similar effect of store mobilisation in mouse sperm from both wild type mice and also in a proportion of sperm from mice null for CatSpers (Marquez et al. 2007).
It appears that stored Ca$^{2+}$ in the neck/midpiece region of human sperm acts similarly. In these cells, treatments that induce Ca$^{2+}$ influx can ‘switch on’ cyclical mobilisation of this store (causing cytoplasmic [Ca$^{2+}$]$_i$ oscillations) apparently due to a form of CICR (Kirkman-Brown et al. 2004, Bedu-Addo et al. 2005, Harper et al. 2005; section Evidence for functional calcium storage in sperm). In many of the cells that show oscillations an increased excursion of the flagellum, often associated with asymmetrical bending of the midpiece, occurs during the [Ca$^{2+}$]$_i$ peaks. Flagellar activity ‘relaxes’ during the intervening troughs (Harper et al. 2004, Bedu-Addo et al. 2005, Machado-Oliveira et al. 2008; Fig. 4). 4-Aminopyridine, an extremely potent inducer of hyperactivation in human sperm (Gunter et al. 2004) causes reversible, repeatable mobilisation of Ca$^{2+}$ stored in the neck/midpiece. In many cells, Ca$^{2+}$ mobilisation is accompanied by (and apparently induces) sustained, asymmetric bending of the proximal flagellum, while, the distal flagellum continues to beat. Upon removal of 4-aminopyridine, [Ca$^{2+}$]$_i$ falls and the flagellar bend ‘relaxes’ (Costello S unpublished data). Investigations of the Ca$^{2+}$ dependence of 4-AP-induced hyperactivation clearly show that, in mouse and bovine sperm, mobilisation of stored Ca$^{2+}$ is sufficient to initiate hyperactivation, but also suggest that store-operated Ca$^{2+}$ influx contributes to maintenance of this mode of motility. Castellano et al. (2003) observed that blockers of store operated channels caused inhibition of motility in human sperm.

**Separation of store-regulated activities**

Ca$^{2+}$ mobilisation from the acrosome and from the store(s) in the sperm neck/midpiece regulate different activities. It is, therefore, important that they can be controlled separately. In mammals acrosome reaction is believed to occur at the surface of the zona pellucida. Sperm-zona pellucida interaction activates signalling cascades leading to acrosomal exocytosis (Florman et al. 2008; Fig. 5). The acrosomal content then disperses slowly (Harper et al. 2008), its content probably aiding penetration of the zona pellucida matrix. Though sperm in the early stages of acrosome reaction may bind to and go on to penetrate the zona pellucida (Buffone et al. 2008), it is likely that those that undergo acrosome reaction prematurely will be severely compromised in their ability to fertilise. It is therefore vital that stimuli that mobilise Ca$^{2+}$ stored in the midpiece/neck region, for regulation of motility, should not ‘accidentally’ activate the acrosomal store. In human sperm stimulated with progesterone, large [Ca$^{2+}$]$_i$ oscillations at the sperm neck and consequent regulation of flagellar activity cause no detectable increase in the occurrence of acrosome reaction (Harper et al. 2004). Also, in an elegant study on hamster sperm, Suarez & Dai (1995) observed that [Ca$^{2+}$]$_i$ had increased to a greater extent in the midpiece than in the head in hyper-activated sperm, while the reverse was true for acrosome-reacted sperm.

How might this be achieved? The store(s) in the sperm neck/midpiece appears to be mobilised by CICR. In human sperm a minimal level of Ca$^{2+}$ influx at the plasma membrane is required to support cyclical Ca$^{2+}$ mobilisation (Ca$^{2+}$ oscillations) but pharmacological blockade of IP$_3$Rs (with 2-APB) or of PLC (with U73122 or neomycin) has no effect. Thus, this activity does not seem to require agonist-stimulated generation of IP$_3$. In fact, after stimulation with progesterone to induce [Ca$^{2+}$]$_i$ oscillations, many cells continue to oscillate after removal of the agonist, presumably because, in these cells, Ca$^{2+}$ ‘leak’ at the plasmalemma can support CICR once it has been initiated (Harper et al. 2004). The putative expression of RyRs in the sperm neck/midpiece (section Ryanodine receptors) is consistent with Ca$^{2+}$ mobilisation by CICR, but why are IP$_3$Rs (section Inositol trisphosphate receptors) also expressed here? First, IP$_3$Rs may play a role in CICR. It is known that these receptors can support this process provided that an adequate ‘background’ level of IP$_3$ is present (Berridge 1993). Such a background level of IP$_3$ may be present in capacitated sperm, particularly since hydrolysis of phosphatidylinositol 4,5-bisphosphate to generate IP$_3$ may be activated by elevation of [Ca$^{2+}$]$_i$ (Thomas & Meizel 1989). Second, during the burst of IP$_3$ generation that follows zona pellucida binding and

![Figure 4](image-url) Mobilisation of stores Ca$^{2+}$ at the sperm neck/midpiece leads to modulation of flagellar activity. (a) Shows phase image of immobilised, Oregon Green BAPTA 1-labelled human sperm. b–g are a series of pseudo coloured fluorescence images (taken at 10 s intervals) of the same cell during a Ca$^{2+}$ transient induced by treatment with progesterone. The cell was bathed in medium with no added Ca$^{2+}$. Ca$^{2+}$ is liberated at the sperm neck and spreads into both the posterior head and the flagellum. During the Ca$^{2+}$ peak a pronounced bend occurs in the proximal flagellum (e) and excursion of the flagellum increases (d and f).
mediates emptying of the acrosomal store, the store in the midpiece/sperm neck may be strongly activated through its IP₃Rs. In addition, zona binding mat activate CatSpers (Xia & Ren 2009; section The acrosomal store) if either or both these processes occur, arrival of the sperm at the zona pellucida will initiate a combination of acrosome reaction and intense hyperactivation (Fig. 5). In this context, it is noteworthy that hyperactivation is intensified in acrosome reacted hamster sperm (induced by zona pellucida) and that in these cells [Ca²⁺]ᵢ is increased in the flagellum (Suarez & Dai 1995).

**Outlook**

Only 10 years ago the presence of Ca²⁺ stores in sperm was a matter for debate (Publicover & Barratt 1999). The presence of these stores is now well established and there is little doubt that they enable the cell to generate Ca²⁺-signals that vary in size, ‘shape’ and location within the cell, permitting discrete control of different Ca²⁺-regulated functions. However, there are many aspects of the activation and control of store mobilisation of which we are still ignorant and on which future work should be focussed.

The identity and characteristics of the store located at the sperm neck/midpiece is far from clear. It is likely that Ca²⁺ storage here comprises more than one structure. Furthermore, the nature of the pumps and channels that are functional in this region is disputed. There is evidence for expression and or function of SERCAs, SPCAs, IP₃Rs and RyRs in the Ca²⁺ stores of the neck/midpiece (see sections Evidence for functional calcium storage in sperm and Ca²⁺ storage at the sperm neck/midpiece) and it may be that these Ca²⁺-handling ‘tools’ are all expressed in this region of the cell but used in discrete ways to regulate functionally separate Ca²⁺ storage compartments.

Another area of great interest is the question of whether Ca²⁺ stores in sperm are functional in freshly ejaculated cells. Is delay of filling of the store(s) or delay of store ‘priming’ (development of sensitivity to stimulation) a mechanism by which premature activation of Ca²⁺-regulated processes is controlled? It has been suggested recently that Ca²⁺ mobilisation from the RNE might be regulated during capacitation by activity of Src kinase which is localised to this region of human sperm and is activated during capacitation (Varano et al. 2008). Furthermore, residence in the female tract may affect sensitivity of Ca²⁺ mobilisation. For instance, NO⁻, which is generated by endothelial cells of the oviduct, sensitises Ca²⁺ mobilisation from the store in the neck/midpiece of human sperm (Machado-Oliveira et al. 2008).

Finally, the relationship between mobilisation of stored Ca²⁺, influx of Ca²⁺ at the sperm plasma membrane, hyperactivation and acrosome reaction must be elucidated. CatSper channels are required for normal hyperactivation of mouse sperm. Cells null for these channels cannot hyperactivate and the mice are sterile (Navarro et al. 2008). More recently they have been implicated in acrosome reaction (see section The acrosomal store). Since stored Ca²⁺, at least at the neck/midpiece of human sperm, can be mobilised by CICR, Ca²⁺-influx through CatSpers may recruit stored Ca²⁺, in addition to Ca²⁺ entering through the plasma membrane (Fig. 5). The observations of Xia et al. (Xia et al. 2007, Xia & Ren 2009) that the elevation of [Ca²⁺]ᵢ, that occurs upon opening of CatSper channels can propagate to the sperm head is consistent with this.
suggestion. Direct pharmacological mobilisation of stored Ca\(^{2+}\) can itself induce hyperactivation in wild type mouse sperm bathed in Ca\(^{2+}\)-free medium and also in a proportion of sperm from mice null for CatSper (Marquez et al. 2007). Thus, store mobilisation alone is apparently sufficient to induce hyperactivation (Fig. 5). An important part of the function of CatSper channels in supporting hyperactivation may be to induce CICR at the sperm neck/midpiece.

Recent findings have revealed unexpected sophistication in the Ca\(^{2+}\) signalling capability of sperm (Publicover et al. 2007). It may be that there is considerably more to functioning of the Ca\(^{2+}\) store in sperm than we currently know.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

**Funding**

L L and C W F were supported by the Wellcome Trust (grant no. 078905), S C and K N were in receipt of BBSRC studentships, G M-O was in receipt of a studentship from Fundação para a Ciência e Tecnologia (FCT) Portugal (SFRH/BD/17780/2004).

**Acknowledgements**

Our thanks to Gordon Milne for his expert help with the electron microscopy.

**References**


Blackmore PF 1993 Thapsigargin elevates and potentiates the ability of progesterone to increase intracellular free calcium in human sperm: possible role of perinuclear calcium. *Cell Calcium* 14 53–60.


Espino J, Mediero M, Lozano GM, Bejarano I, Ortiz A, García JF, Pariente JA & Rodríguez AB 2009 Reduced levels of intracellular calcium releasing in spermatozoa from asthenozoospermic patients. *Reproductive Biology and Endocrinology* 7 11.


436 S Costello and others

Kim MS, Zeng W, Yuan JP, Shin DM, Worley PF & Muallem S

Herrick SB, Schweissinger DL, Kim SW, Bayan KR, Mann S & Cardullo RA


Jeong SY & Seol DW 2008 The role of mitochondria in apoptosis. BMB Reports 41 11–22.


Lanini L, Bach O & Carafoli E 1992 The calcium pump of the liver nuclear membrane is identical to that of the endoplasmic reticulum. Journal of Biological Chemistry 267 11548–11552.


Marquez B, Ignott G & Suarez SS 2007 Contributions of extracellular and intracellular Ca\(^{2+}\) to regulation of sperm motility: release of intracellular stores can hyperactivate CatSper1 and CatSper2 null sperm. Developmental Biology 303 214–221.


Michelangeli F, Mezma N, Tovey S & Sayers LG 1995 Pharmacological modulators of the inositol 1,4,5-trisphosphate receptor. Neuropharmacology 34 1111–1122.


O’Toole CM, Arnoult C, Darzon A, Steinhardt RA & Florman HM 2000 Ca\(^{2+}\) entry through store-operated channels in mouse sperm is initiated by egg ZP3 and drives the acrosome reaction. Molecular Biology of the Cell 11 1571–1584.


Tomes CN, McMaster CR & Saling PM 1996 Activation of mouse sperm phosphatidylinositol-4,5 bisphosphate-phosphohydrolase C by zona pellicula is modulated by tyrosine phosphorylation. Molecular Reproduction and Development 43 196–204.


Wootton LL & Michelangeli F 2006 The effects of the phenylalanine 256 to valine mutation on the sensitivity of sarcoplasmic/endoplasmic reticulum Ca2+ ATPase (SERCA) Ca2+ pump isoforms 1, 2, and 3 to thapsigargin and other inhibitors. Journal of Biological Chemistry 281 6970–6976.


Received 11 April 2009
First decision 20 May 2009
Accepted 19 June 2009