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A Model for the Acrosome Reaction in Mammalian Sperm

Julie Simons[1] · **Lisa Fauci**[2]

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Abstract

The acrosome reaction is a complex, calcium-dependent reaction that results in an exocytotic event required for successful fertilization of the egg. It has long been thought that the acrosome reaction occurs upon sperm binding to the zona pellucida, a viscoelastic layer surrounding the oocyte. Recent studies have suggested that the reaction may even occur before the sperm encounters the zona, perhaps mediated by progesterone or some other agonist. It has been particularly difficult to understand differences between progesterone-induced and zona-induced reactions experimentally and whether one substance is the more biologically relevant trigger. Until this present work, there has been little effort to mathematically model the acrosome reaction in sperm as a whole. Instead, attention has been paid to modeling portions of the pathways involved in other cell types. Here we present a base model for the acrosome reaction which characterizes the known biochemical reactions and behaviors of the system. Our model allows us to analyze several pathways that may act as a stabilizing mechanism for avoiding sustained oscillatory calcium responses often observed in other cell types. Such an oscillatory regime might otherwise prevent acrosomal exocytosis and therefore inhibit fertilization. Results indicate that the acrosome reaction may rely upon multiple redundant mechanisms to avoid entering an oscillatory state and instead maintain a high resting level of calcium, known to be required for successful acrosomal exocytosis and, ultimately, fertilization of the oocyte.

Keywords Acrosome reaction · Mathematical model · Calcium signaling · Biphasic response

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✉ Julie Simons
jsimons@csum.edu

Lisa Fauci
fauci@tulane.edu

[1] Department of Sciences and Mathematics, California Maritime Academy, 200 Maritime Academy Dr., Vallejo, CA 95490-8181, USA

[2] Department of Mathematics and Center for Computational Science, Tulane University, 6823 St. Charles Ave., New Orleans, LA 70118, USA

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1 Introduction

Successful fertilization of the egg involves many biomechanical and biochemical changes of the sperm as it traverses the oviduct and reaches the cumulus–oocyte complex. The acrosome reaction (AR) is a calcium-dependent reaction that is one significant part of this process. An acrosome-reacted sperm releases the contents of a secretory vesicle known as the acrosome, located in the anterior portion of the sperm head. The acrosome may contain enzymes that aid in penetration of the extracellular matrix surrounding the oocyte, part of which is a secretion known as the zona pellucida (ZP) (Breitbart 2002; Florman 1994). Further, the release of this vesicle may enable the spermatozoa to attach to the ZP via either the now exposed inner acrosomal membrane or plasma membrane (Yanagimachi and Phillips 1984). The acrosome reaction itself involves several known calcium pathways common to other cellular processes. While the acrosome reaction must occur for a sperm to successfully fertilize the egg, the precise role it plays in fertility, as well as the biochemical signaling cascade itself, is still under investigation.

There are two primary proposed physiologically relevant triggers for this reaction in vivo, the zona pellucida glycoprotein (ZP3 in humans) and progesterone (Florman et al. 2008; O'Toole et al. 2000; Harper et al. 2006). Both of these agonists can induce the AR in laboratory settings. Until recently, zona pellucida glycoproteins were thought to be the more biologically relevant agonist, as it was thought that the AR happens when the sperm binds to the egg where the ZP glycoproteins are located in successful fertilization events. It is well documented that the ZP can trigger or accelerate the acrosome reaction (Abou-haila and Tulsiani 2009; Bleil and Wassarman 1983; Buffone et al. 2009; Cherr et al. 1986; Crozet and Dumont 1984; Florman and Storey 1982; Tollner et al. 2003; Uto et al. 1988). However, recent in vivo mouse studies have suggested that the acrosome reaction is often initiated before reaching the ZP (Hino et al. 2016; Inoue et al. 2011; Jin et al. 2011; Spina et al. 2016), indicating that ZP-induced acrosome reactions may not be the primary mechanism in vivo. Whether or not progesterone specifically is a primary inducer is still a subject of debate, but this theory relies upon the fact that progesterone is secreted by the cumulus cells surrounding the oocyte and the cumulus is well known to induce the AR (Jin et al. 2011; Yanagimachi 1994).

The AR involves a characteristic calcium (Ca^{2+}) response in the head of the sperm. Calcium is a ubiquitous component in signaling and regulation of various cellular processes across species. Calcium oscillations have long been implicated in other secretory events, such as neurotransmitter synaptic release processes (Neher and Sakaba 2008; Rubin 1970). In sperm, the acrosome reaction is not a periodic release process but an end-stage event before fusion of the sperm with the plasma membrane of the egg. This reaction is characterized by a biphasic calcium response: an initial sharp rise in calcium concentrations in the head of the sperm followed by a relaxation phase where the calcium concentration tends toward a steady, elevated level.

In this paper, we develop a mathematical model of the acrosome reaction in mammalian sperm that can serve as a tool to understand the importance of various components of the reaction and contrast this with previous calcium models in other cell types. In the following sections, we will describe the relevant biology and reactions believed to be involved and then introduce a base model that reproduces the biphasic calcium response characteristic of the AR. Because many of the components of the acrosome reaction pathway have been studied in other biological contexts, we base our model on previous mathematical models for calcium dynamics developed for different systems (for an overview, see Keener and Sneyd 2009). Our model focuses on the temporal dynamics of the reaction in order to match to non-spatial data from experiments, where often calcium levels are recorded simply as a change in concentration in the entire sperm head. We then investigate an extension of this model which more accurately describes the components that have been experimentally implicated in the full reaction. We also address experimental results in which slow oscillations in calcium have been observed in human sperm (Kirkman-Brown et al. 2004; Sánchez-Cárdenas et al. 2014), and discuss the implications this has from both a mathematical and a biological perspective.

2 Biological Behavior

In this section, we introduce the general components of the acrosome reaction pathway which will be relevant to constructing the model. The sperm head consists of a sperm nucleus, a cytoplasmic compartment and the acrosome, all held within the plasma membrane of the sperm (see Fig. 1). In physiologically relevant conditions, either a zona pellucida glycoprotein (identified as ZP3 in human sperm) or another chemical signal (such as progesterone) initiates the acrosome reaction by binding to a

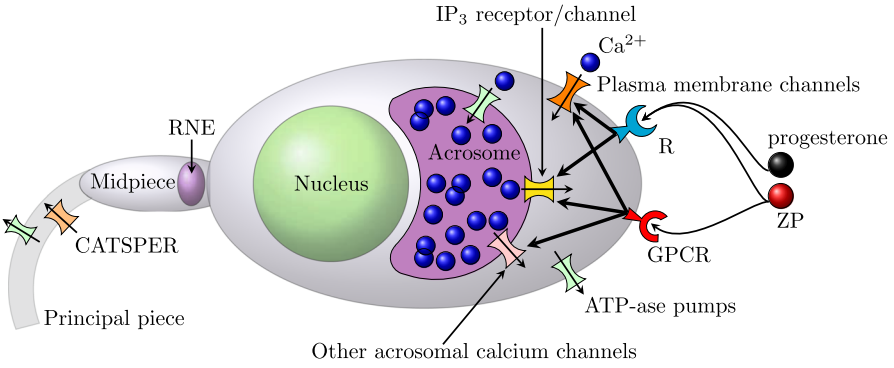


Fig. 1 A depiction of the sperm head and the acrosome reaction response to stimulation from zona pellucida glycoprotein (ZP) or another signal such as progesterone. Direction of calcium flow through channels is denoted by small arrows, and stimulation of channels is shown by thicker black arrows from receptors (R or GPCR). GPCR stands for G-protein-specific receptor, whereas R represents a pertussis toxin-insensitive receptor. Ca^{2+}-ATP-ase pumps are shown in green, IP_3 receptors/channel is yellow, outer membrane channels (plasma membrane, CATSPER) are orange, and other acrosomal calcium channels are pink. Arrows through pumps denote transport of calcium. Drawing is not to scale (Color figure online)

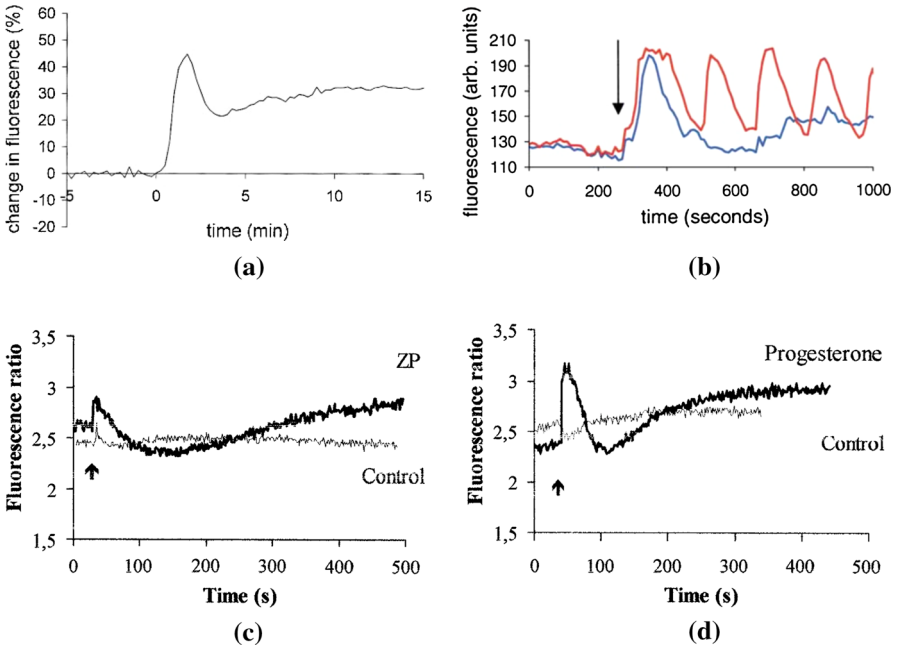


Fig. 2 Experimentally measured calcium responses in the sperm head, after stimulation by progesterone or zona pellucida glycoproteins (ZP). **a** Average calcium concentration measured in sperm heads after stimulation by progesterone, reproduced with permission from Kirkman-Brown et al. (2000). **b** Calcium concentrations measured in the sperm head after stimulation by progesterone at time indicated by black arrow, showing both oscillatory and non-oscillatory behavior. This figure was originally published in Kirkman-Brown et al. (2004). **c** Calcium response in a single sperm head after stimulation from zona pellucida glycoproteins at time indicated by black arrow, reproduced with permission from Patrat et al. (2000). **d** Calcium response in a single sperm head after stimulation from progesterone at time indicated by black arrow, reproduced with permission from Patrat et al. (2000) (Color figure online)

receptor on the plasma membrane of the sperm head. Receptor binding then activates at least one of two enzymes, which may then result in the opening of calcium channels along the plasma membrane or on the acrosomal membrane, causing calcium entry into the cytoplasm. These calcium channels themselves may exhibit calcium-induced calcium release (CICR) behavior, which serves to further elevate calcium levels in the cytoplasm (Roderick et al. 2003).

The result of this process is referred to as biphasic response, indicating an initial sharp increase in calcium, followed by a relaxation phase toward a sustained elevated resting level of calcium. In Fig. 2a, we show an average biphasic calcium response of sperm heads after being stimulated by progesterone, reproduced from Kirkman-Brown et al. (2000). In Fig. 2b, we show the calcium response of two different sperm heads after being stimulated by progesterone, one showing a biphasic response (blue) and another showing slow calcium oscillations (red), reproduced from Kirkman-Brown et al. (2004). In Fig. 2c, d, we show similar biphasic calcium responses of sperm after being stimulated by zona pellucida glycoproteins and progesterone, respectively, reproduced from Patrat et al. (2000).

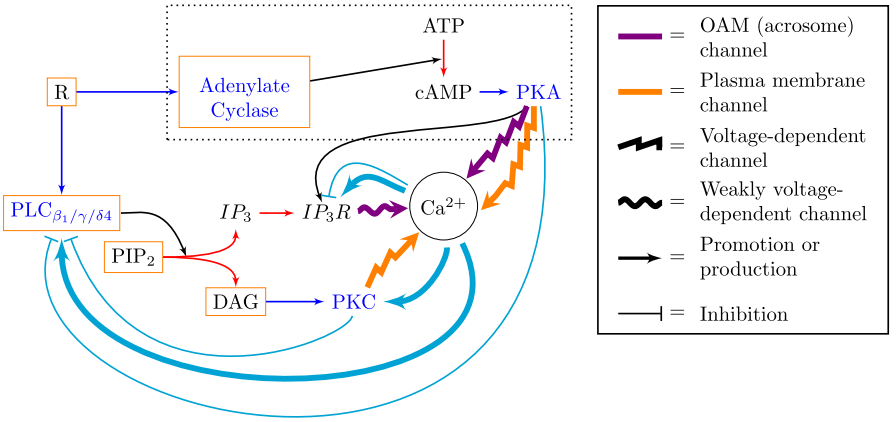


Fig. 3 Diagram representing the various components of the acrosome reaction. *R* represents either a G-protein-coupled receptor (known to be pertussis toxin sensitive) or a pertussis toxin-insensitive receptor. The reaction can be initiated by either zona pellucida glycoproteins or another signal such as progesterone. The dotted box corresponds to a part of the pathway that is G-protein pathway-specific. IP_3R denotes the IP_3 receptor located on the acrosomal membrane. Black arrows correspond to promotion or enzymatic activity, red arrows correspond to binding or creation (i.e., "A turns into B"), and dark blue arrows correspond to activation of enzymes. Enzymes are denoted by blue text, and orange boxes denote a location on/near the plasma membrane (Color figure online)

The specific reactions involved in the acrosome reaction are still an active area of investigation. Figure 1 depicts the physical components of the acrosome reaction, while Fig. 3 shows an overview of the specific reactions thought to be involved in the acrosome reaction. Stimulation of the acrosome reaction requires activation of plasma membrane channels, which may include voltage-operated calcium channels, TRPC channels and poorly selective cation channels (see Arnoult et al. 1999, Arnoult et al. 1996, Evans and Florman 2002).

Two classes of receptors on the plasma membrane of the sperm have been hypothesized to be involved in the initiation of the acrosome reaction. One is a G-protein-coupled (pertussis toxin-sensitive) receptor, which would lead to the activation of two enzymes, known as phospholipase C (PLC) and adenylate cyclase (AC) (Bastiaan et al. 1999; Florman et al. 1989). Another is a pertussis toxin-insensitive receptor, which could also lead to a PLC response and may also activate a plasma membrane calcium channel (Tesarik et al. 1996). The G-protein receptor pathway has only been shown to be active when ZP is present, whereas both ZP and progesterone seem to bind to a pertussis toxin-insensitive receptor. It has been hypothesized that the G-protein receptor pathway would be triggered by the ZP glycoprotein and lead to an initial, relatively small rise in calcium via AC, which could then indirectly stimulate PLC and lead to a secondary increase in calcium concentration (Breitbart and Spungin 1997; Broad et al. 2001). In summary, a G-protein-coupled receptor can be activated by ZP, but a pertussis toxin-insensitive receptor may be activated by ZP or progesterone.

AC is an enzyme involved in transforming adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP). It is known that this could enable calcium release from the acrosome by activating an acrosomal calcium channel directly or indirectly

via protein kinase A (PKA) (Patrat et al. 2000; Spungin and Breitbart 1996). PKA has been shown to both stimulate and inhibit inositol 1,4,5-trisphosphate (IP_3) receptor calcium release (Bird et al. 1993; Bruce et al. 2003; Nakade et al. 1994; Tertyshnikova and Fein 1998). AC may also be sensitive to calcium concentration, as in other systems (Eliot et al. 1989).

Several isoforms of phospholipase C ($PLC_{\beta_1/\gamma/\delta 4}$) may be involved in the reaction, but PLC is necessary for ZP-induced acrosome reactions (Fukami et al. 2001) and PLC activity can be modulated by a wide class of receptors, including pertussis toxin-insensitive receptors (Cockcroft and Thomas 1992). One role of PLC is to cleave phosphatidylinositol 4,5-bisphosphate (PIP_2) into diacylglycerol (DAG) and IP_3. IP_3 can bind to IP_3 receptors which have been localized to the acrosome (Walensky and Snyder 1995), but have also been shown to be present in the redundant nuclear envelope (RNE) in the neck region of the sperm (Ho and Suarez 2001, 2003). These IP_3 receptors control the opening of an IP_3-gated channel. This channel can release calcium from an internal store such as the acrosome or RNE into the cytoplasm. IP_3 receptor channels are weakly voltage dependent and known to exhibit both calcium-induced calcium release and long-term calcium inhibition (Atri et al. 1993; Keener and Sneyd 2009). Additionally, the production of DAG from PIP_2 can activate protein kinase C (PKC), which may be able to open a voltage-dependent plasma membrane calcium channel, thereby increasing cytosolic calcium levels (Breitbart et al. 1992; Spungin and Breitbart 1996).

Besides long-range calcium inhibition of IP_3-gated calcium channels on the acrosome, there are at least two additional feedback mechanisms that may be present in this system. The first is due to the involvement of protein kinases, which have been shown to have an inhibitory effect upon PLC. In particular, both PKC and PKA have been shown to have inhibitory effects on the activity of PLC (Premack and Gardner 1992; Rhee et al. 1992; Yue et al. 2000). Secondly, PLC requires calcium for its activity, indicating that an increase in calcium could stimulate PLC activity (Premack and Gardner 1992). This would lead to further calcium release.

There are other potential sources of calcium which may have an effect upon calcium levels in the sperm head. CATSPER channels are located along the principle piece of the sperm flagellum (see Fig. 1) and may propagate calcium signals toward the head of the sperm (Xia et al. 2007). In the midpiece, the redundant nuclear envelope (RNE) is another internal store which may have both ATP-ase pumps and IP_3 receptors (not shown in Fig. 1) regulating its calcium content (Ho and Suarez 2001, 2003). It is possible that calcium may be transported from the RNE to the sperm head.

In the next section we will focus on developing a base model for the acrosome reaction which can exhibit a simple biphasic response from binding to a generic agonist such as ZP or progesterone. We will then investigate how incorporating more detail in our base model may better resolve the type of experimental behavior shown in Fig. 2. Lastly, we will discuss the biological implications and why slow calcium oscillations could occur in some settings.

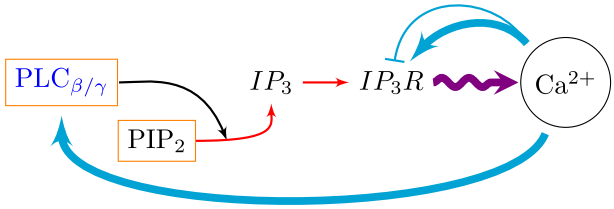


Fig. 4 IP_3 pathway in isolation (Color figure online)

3 Mathematical Model

In order to develop a base mathematical model that can exhibit a biphasic response, we first introduce the following notation to describe the concentrations of species we will consider:

$$L_c(t) = [\text{PLC}], \qquad I(t) = [IP_3], \qquad R_I(t) = [IP_3 \text{ receptor activity}],$$
$$C(t) = [\text{Ca}^{2+}], \qquad D(t) = [\text{DAG}], \qquad P_{kc}(t) = [\text{PKC}],$$

For simplicity, we will assume ATP is abundant in the sperm head so there is no need to explicitly track its concentration. We will also model agonist binding as a simple on–off switch. This activation will correspond to activating phospholipase C. One could make the receptor response more dynamic, but we choose to focus on the downstream kinetics of the acrosome reaction.

3.1 Base Model

For the base model, we leave out the effects of the protein kinases and focus on just the IP_3 pathway depicted in Fig. 4. We will assume a calcium-IP_3 receptor model based on Atri et al. (1993), Sneyd et al. (1995) with the IP_3 concentration model described in Keizer and Young (1992). We add a PLC (L_c) dependence for IP_3 production, resulting in Eqs. (1) through (4):

$$\frac{dC}{dt} = -\frac{k_1 C^2}{K_1^2 + C^2} + k_2 R_I + k_6 \tag{1}$$

$$R_I = \frac{I^n}{I^n + K_2^n}\left(k_3 + \frac{(1-k_3)C}{K_3 + C}\right)h(t) \tag{2}$$

$$\frac{dI}{dt} = \frac{k_4 L_c C}{K_4 + C} - k_5 I \tag{3}$$

$$\tau \frac{dh}{dt} = \frac{\kappa^2}{\kappa^2 + C^2} - h \tag{4}$$

where n, τ, κ, k_i and K_i are parameters (for $i = 1, \ldots, 6$). Each K_i has units of concentration and corresponds to a half-maximum value (the concentration at which the term is half its maximum value). The parameters k_1, k_2, k_4 and k_6 have units of

concentration per time and can be thought of as production or degradation rates. The k_5 parameter is in units of s^{-1} and controls the rate of degradation of IP_3. The parameter τ has units of time and controls the timescale of feedback and is taken to be large due to the slow timescales of calcium dynamics in sperm. The parameter n is an integer that sets the functional form of the IP_3 receptor response to IP_3 binding. Lastly, k_3 is a unitless parameter between 0 and 1 that controls the calcium dependence of the IP_3 receptor. For instance, if $k_3 = 1$, the IP_3 receptor is not calcium-dependent at all. If $k_3 = 0$, the receptor requires calcium to be able to release calcium.

This IP_3 receptor model is able to capture the effects of CICR and calcium inhibition of the IP_3-gated calcium channel on the acrosomal membrane. The three terms on the right-hand side of Eq. (1) imply that (a) calcium is pumped out of the cytosol with a Hill functional response, characteristic of ATP-ase pumps known to pump calcium across membranes; (b) calcium is released through the IP_3-gated channel R_I; and (c) there is a constant leak of calcium into the sperm head. In our scenario, we have two membranes of interest (the plasma membrane and the acrosomal membrane), but since we are excluding spatial effects, we will not differentiate between ATP-ase pump locations. Experimental evidence for a constant leak in calcium, modeled by k_6, was shown in Wennemuth et al. (2003), and the acrosome reaction has been shown to require external calcium in several experimental assays (Rossato et al. 2001; Spungin and Breitbart 1996). The value of k_6 will be chosen such that it results in a resting intracellular calcium level of 0.1 μM, to match experimental evidence that resting levels are around 0.1–0.25 μM (Wennemuth et al. 2003).

Equation (2) is a model for the IP_3-gated channel R_I. This channel has a Hill-type response to IP_3 concentration, is calcium-dependent and requires a slow inhibitory response modeled by $h(t)$ in Eq. (4). The activity of R_I saturates at high concentrations of calcium or IP_3, and the extent of calcium dependence of the channel is set by the parameter k_3. The inhibitory response is modeled by a *decrease* in $h(t)$, which will occur more strongly if calcium levels are elevated. In particular, Eq. (4) has a production term that decreases with increasing calcium concentration and has a slower timescale (set by the parameter τ) than the other equations. The parameter τ is found by matching timescales to experimental data.

In Eq. (3), IP_3 has one source term involving PLC (L_c) and one constant degradation term. PLC activity will be prescribed by simply setting $L_c = 1$ when it is activated and 0 when it is inactive. We note that all forms of phospholipase C require calcium for activation and calcium has been shown to activate PLC (Thore et al. 2005). Instead of incorporating calcium dependence of PLC activity directly, we include the calcium dependence in Eq. (3) for IP_3 (as in Keizer and Young 1992) and the IP_3 receptor (as in Sneyd et al. 1995). In all simulations, we set $L_c = 0$ initially to demonstrate the equilibrium resting values before receptor stimulation and L_c activation. In this initial phase, $IP_3 = 0$, IP_3 receptors will be closed, and calcium will remain at its resting level.

There are two scalings that we are concerned with matching experimentally: the first is the calcium concentrations themselves. Upon initiating the acrosome reaction, we aim to observe a change in calcium concentration of 10–50% for the initial, sharp elevation and a sustained response of around 25-30% above the basal calcium levels before stimulation (to match to experimental data such as the data shown in Fig. 2).

The other scaling we aim to match is the timescale of the process. Figure 2 indicates that the initial rise in calcium occurs within 1–2 min of stimulation, the subsequent decreasing phase occurs in the next 1–2 min, and the final elevation phase occurs within a 5–10-min range thereafter.

With this in mind, we now present representative behavior from this model for three different parameter regimes listed in Table 1. In parameter Set 1, we choose typical (or intermediate) values for all parameters in the model based on existing parameter estimates from other models (for other cell types). Figure 5a shows the resulting behavior for this parameter set, which also gives a sustained calcium elevation of 25%. For this choice of parameters, we only observe a monotonic response in calcium, IP_3, and IP_3 receptor activity R_I.

We also observe two other types of behavior from this model, an oscillatory response and a biphasic response (essentially a damped oscillatory response). For the biphasic response, we have chosen the model parameters (Set 2) such that we can closely approximate both the experimental timescale and the appropriate levels of changes in calcium concentration. In this case, we reproduce the types of behavior of the curves in Fig. 2, with a sharp peak followed by a decline, and then *increase* toward a higher resting calcium level. This is shown in Fig. 5b, where the initial increase in calcium is about 48% and the final calcium concentration is approximately 24% above the initial resting rate, before stimulus. With slight parameter changes, this same model can exhibit the oscillatory response shown in Fig. 5c, which gives a similar temporal oscillation to the data from Kirkman-Brown et al. (2000), shown in Fig. 2. Our concentration scales show sustained oscillations of calcium in the 0.18–0.79-μM range, which is significantly higher than the range of 0.11–0.15 μM we obtain from normalizing the data from Kirkman-Brown et al. (2000) to have a resting calcium level of 0.1 μM. However, this range of oscillatory behavior is within biologically realistic levels for other cells types (for instance, see Cuthbertson and Chay 1991).

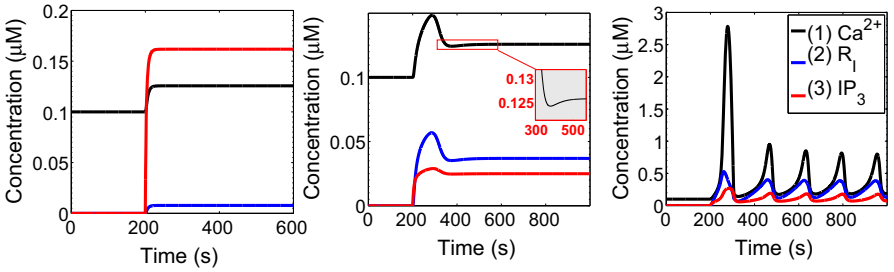
This model can capture the type of calcium behaviors observed experimentally. The parameter regimes for the monophasic and oscillatory responses we have shown are within biologically relevant parameter ranges. We note that IP_3 has been shown to be elevated in the initial stages of the acrosome reaction (Thomas and Meizel 1989), but IP_3 levels have not been tracked for experiments in which calcium oscillations have been reported. Thus, while our model is consistent with calcium observations, further experiments are required to check whether IP_3 oscillations are indeed accompanying calcium oscillations.

The different parameter sets listed in Table 1 highlight some of the important parameters in our model. For instance, there are changes in orders of magnitude for k_2, k_4, K_2, K_3 and τ. These indicate that *decreases* in calcium release through the IP_3-gated channels via k_2 or *decreases* in IP_3 production via k_4 can enable oscillatory behavior. For the parameter K_2, a decrease would mean a lower half-activation constant for IP_3 receptor activity, making the response in R_I stronger, which may enable oscillatory behavior as well. The parameter K_3 does not show a clear correlation with oscillatory behavior. Lastly, because τ is a parameter that is generally set to match experimental data, we note that the timescales of oscillations observed in sperm in Kirkman-Brown et al. (2004) are slow compared to most other cells, so these magnitudes are expected.

Table 1 Parameter estimates for the base model in Eqs. (1)–(4)

	Unit	Values			Reference Values	Source
		Set 1	Set 2	Set 3		
k_1	μM/s	0.75	0.47	0.4376	0.5, 0.75	Keizer and Young (1992), Hong et al. (2008)
k_2	μM/s	8	0.81	0.864	3, 8.1	Sneyd et al. (1995), Atri et al. (1993)
k_3	Unitless	0.11	0.11	0.11	0.11	Atri et al. (1993), Parys et al. (1992), Sneyd et al. (1995)
k_4	μM/s	0.71	0.07	0.0756	0.05, 0.22–0.71, 3	Höfer et al. (2002), Keizer and Young (1992), Young and Keizer (1992); Hong et al. (2008)
k_5	s^{-1}	0.49	0.2241	0.2241	0.066, 0.08, 0.17, 2, 2.5	Horowitz et al. (2005), Höfer et al. (2002), Berridge (1987), Keizer and Young (1992), Hong et al. (2008)
k_6	μM/s	0.15	0.3760	0.0875	0.3	Wennemuth et al. (2003)
K_1	μM	0.2	0.05	0.2	0.09, 0.2	Keizer and Young (1992), Hong et al. (2008); Camello et al. (1996)
K_2	μM	0.5	0.062	0.065	0.01, 0.026, 1.6	Sneyd et al. (1995), Meyer and Stryer (1988), Walensky and Snyder (1995)
K_3	μM	0.7	0.01	0.7	0.7	Atri et al. (1993)
K_4	μM	1	0.7	0.7	0.3,0.7,1.1, 2	Höfer et al. (2002), Atri et al. (1993), Keizer and Young (1992), Spungin and Breitbart (1996)
κ	μM	0.7	0.17	0.7	0.7	Atri et al. (1993), Parys et al. (1992), Sneyd et al. (1995)
n		3	3	3	3	Sneyd et al. (1995)
τ	s	0.2	80	38	0.2	Sneyd et al. (1995)

Parameter Set 1 gives a monotonic calcium response, whereas Set 2 gives a biphasic response, and Set 3 results in calcium oscillations



(a) Parameter Set 1. **(b)** Parameter Set 2. **(c)** Parameter Set 3.

Fig. 5 Example of behaviors observed in model defined by Eqs. (1)–(4). PLC is activated (L_c is set to 1) at 200 s, with parameter values from Table 1. The legend in the upper right panel corresponds to all plots. Parameter Set 1 shows a simple monotonically increasing calcium response. Parameter Set 2 (**b**) depicts the closest behavior to the acrosome reaction calcium responses, while parameter Set 3 captures the oscillatory type of behavior observed in some human sperm. See Fig. 2 as a comparison (Color figure online)

Bifurcation Analysis These parameters and range of behaviors suggest a bifurcation analysis to determine when parameter changes would change the system between oscillatory and stable. While many parameters could be chosen, we choose to bifurcate using the quantity $k_4 L_c$, because this could be tested experimentally by modulating the activity of PLC using either activating or inhibiting agonists. Figure 6 shows the results of a bifurcation analysis (performed using AUTO Doedel et al. 2007) on this systems using the parameter k_4, revealing that this model has two Hopf bifurcations. A Hopf bifurcation is characterized by having one equilibrium solution which gives way to a periodic solution as a parameter in the model is changed. This should be thought of as performing an analysis on the behavior of the system if we increase the concentration of activated phospholipase C (L_c) from zero. Thus, for an intermediate range of activated phospholipase C (corresponding to $k_4 L_c$ between 0.08 and $0.14\,\mu\mathrm{M\,s^{-1}}$), we observe oscillatory behavior. Outside of this range the model has a stable fixed point, which is the final calcium concentration that will be achieved over time. This level corresponds to the elevated calcium level observed toward the end of acrosome reaction experiments, before exocytosis occurs. The calcium levels observed are physiologically relevant, though the oscillatory levels do not correspond to the same levels observed experimentally.

Because this model is lacking certain biological mechanisms known to be a part of calcium regulation—such as protein kinase activity—in the acrosome reaction, we do not expect to match experimental data for the biphasic and oscillatory calcium responses precisely on every scale (temporal, relative calcium response levels and shapes of response curves). In the interest of understanding the contributions of various other pathways that have been implicated in the acrosome reaction, we now focus on extending this base model to include other components of the full pathway.

3.2 Incorporating PKC Feedback

While it has been shown that progesterone does stimulate PKC activity in sperm, there has been experimental evidence both supporting and refuting the involvement of PKC

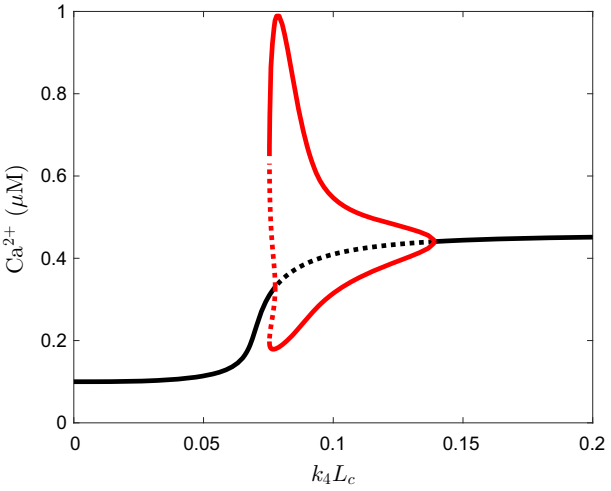


Fig. 6 Bifurcation diagram using parameters from the oscillatory data set in Table 1. By using the parameter $k_4 L_c$ as a bifurcation parameter (recall L_c is set to zero when phospholipase C is inactive), this is equivalent to varying the concentration of activated phospholipase C in the cell. There are two Hopf bifurcation points in this system, where the red lines signify oscillatory states and black lines signify the fixed point of system (1)–(4). Stable states are represented by solid lines, and unstable states are dashed lines. Diagram created using AUTO (Doedel et al. 2007) (Color figure online)

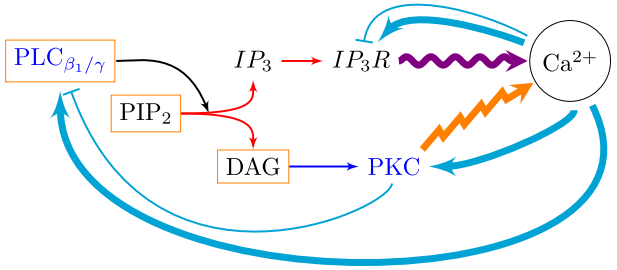


Fig. 7 Diagram representing the IP_3-PKC pathway (Color figure online)

in calcium elevations in the acrosome reaction (Bonaccorsi et al. 1998; Foresta et al. 1995). PKC is a known downstream component of diacylglycerol and has been shown to modulate calcium activity in other contexts (for instance, see Lawrie et al. 1993), so here we extend our model to include PKC activity and understand what effects this could have upon the acrosome reaction.

PKC may be capable of activating a plasma membrane calcium channel as well as having an inhibitory effect on PLC. Our reaction diagram is shown in Fig. 7. We could use the approach of Cuthbertson and Chay (1991) by setting the DAG concentration equal to the IP_3 concentration response, which is assumed because IP_3 and DAG are created from the cleavage of PIP_2. However, this simplification relies upon two assumptions: (1) IP_3 does not bind to its receptor explicitly in the model (in other words, its concentration does not change due to IP_3 receptor dynamics) and (2) the degradation rates of IP_3 and DAG in the cell are the same. While the first assumption is

perhaps biologically acceptable (this requires that the number of IP_3 receptor-binding sites is small compared to the amount of available IP_3), the degradation rates being equivalent are probably an over simplification. We instead choose separate degradation rates that will allow us to explore a wider range of behavior in our model. Thus, we will have two more equations (for DAG and PKC) and several more terms to add to the previous equations, in order to model such responses:

$$\frac{dC}{dt} = -\frac{k_1 C^2}{K_1^2 + C^2} + k_2 R_I + k_6 + k_7 P_{kc} \tag{5}$$

$$R_I = \frac{I^n}{I^n + K_2^n}\left(k_3 + \frac{(1-k_3)C}{K_3 + C}\right)h(t) \tag{6}$$

$$\frac{dI}{dt} = \frac{k_4 L_c C}{K_4 + C}(1 - P_{kc}) - k_5 I \tag{7}$$

$$\tau \frac{dh}{dt} = \frac{\kappa^2}{\kappa^2 + C^2} - h \tag{8}$$

$$\frac{dD}{dt} = \frac{k_4 L_c C}{K_4 + C}(1 - P_{kc}) - k_8 D \tag{9}$$

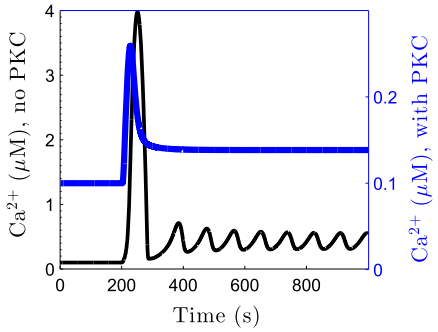
$$\frac{dP_{kc}}{dt} = k_9\left(\frac{D}{K_9 + D}\frac{C}{K_{10} + C} - P_{kc}\right) \tag{10}$$

Equation (5) is similar to Eq. (1), except for the last term modeling the potential for direct stimulation of calcium channels by PKC. Equations (6) and (8) remain unchanged from base model Eqs. (2) and (4). Equation (7) is modified to adjust for PKC inhibition on the activity of PLC. Equation (9) takes the same form of Eq. (7), since IP_3 and DAG with the exception of having a different degradation rate. Lastly, Eq. (10) models PKC activity phenomenologically as a quantity between 0 and 1. The timescale of its activation and deactivation is given by k_9. PKC activity is dependent on DAG and calcium and saturates with high concentrations of both of these molecules.

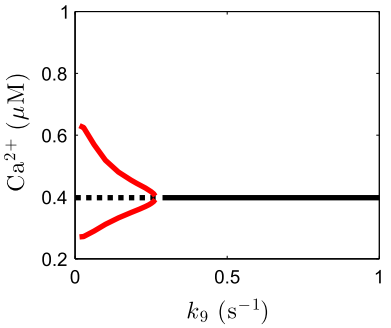
The new parameters in this model again follow the convention that K_9 and K_{10} have units of concentration and correspond to half-maximum values. The parameter k_7 has units of concentration per time and can be thought of as a rate of calcium influx from direct PKC stimulation of a plasma membrane channel (see Fig. 7). The k_8 and k_9 parameters have units of s^{-1} and set the rate of degradation of DAG and production of PKC, respectively.

Including PKC in the reaction pathway causes a damped oscillatory (biphasic) response in a parameter regime that would otherwise result in oscillations, as shown by comparing the blue and black curves in Fig. 8a. Parameter choices are listed in Table 2 and are consistent with experimental measurements. For simplicity, we chose $k_7 = 0$ because PKC-modulated release of calcium does not appear to significantly affect behavior of the system.

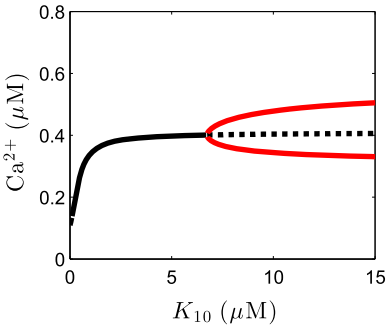
PKC Bifurcation Analysis We also performed a bifurcation analysis on three parameters, which all indicate Hopf bifurcations in this model. These particular bifurcation parameters were chosen because they represent limiting scenarios in which the PKC



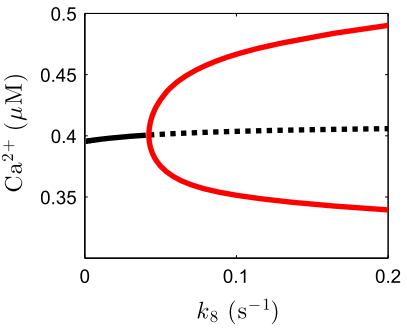
(a) Calcium responses over time.



(b) Bifurcation diagram on k_9.



(c) Bifurcation diagram on K_{10}.



(d) Bifurcation diagram on k_8.

Fig. 8 Calcium responses for the extended model given by Eqs. (5)–(10). Panel (**a**) shows an example of the damping effect of PKC (blue curve) in comparison with the base model from Eqs. (1)–(4) without PKC (black curve). Parameters are from an oscillatory regime in the base model (Set 3 in Table 1, but with $k_4 = 0.1$, with extended model parameters listed in Table 2). In panels (**b**)–(**d**), we use k_8, k_9 or K_{10} as bifurcation parameters to explore the impact of the PKC dynamics upon the calcium response. There are Hopf bifurcation points in all bifurcation parameters. The red lines signify oscillatory states, and black lines signify the fixed point of system (5)–(10), where stable states are solid and unstable states are dashed lines. Faster timescales ($k_9 > 0.28\ \mathrm{s}^{-1}$) of PKC production and degradation, lower calcium half-activation levels ($K_{10} < 6.7\,\mu\mathrm{M}$) and slower degradation of DAG ($k_8 < 0.5\ \mathrm{s}^{-1}$) all result in damping of the oscillatory behavior. Bifurcation diagrams created using AUTO (Doedel et al. 2007) (Color figure online)

Table 2 Additional parameter estimates for new parameters in the extended PKC model from Eqs. (5)–(10)

	Unit	Value	Reference values	Source
PKC				
k_7	$\mu\mathrm{M}\,\mathrm{s}^{-1}$	0	–	–
k_8	s^{-1}	0.013	0.013, 4	Horowitz et al. (2005), Cuthbertson and Chay (1991)
k_9	s^{-1}	5	5	Cuthbertson and Chay (1991)
K_9	$\mu\mathrm{M}$	0.4	0.4	Cuthbertson and Chay (1991)
K_{10}	$\mu\mathrm{M}$	0.1	5–20, 0.1	Kishimoto et al. (1980), Cuthbertson and Chay (1991)

model would reduce to our base model. In this way, we can show how adding this new pathway can stabilize otherwise oscillatory dynamics. Thus, we start with parameters from our base model that would result in oscillations and then add the PKC components gradually by changing the value of bifurcation parameter. For instance, k_9 is a parameter controlling the rate of PKC dynamics, and when this is larger, oscillations can be damped, as shown in Fig. 8b. As k_9 tends toward 0, we recover the base model and oscillations. Additionally, decreasing K_{10} (the half-activation calcium concentration for PKC) or decreasing the degradation rate of DAG using parameter k_8 will damp oscillations in the system, as shown in Fig. 8c and c. An alternative way of viewing this is that as K_{10} or k_8 increases toward infinity, we again recover the base model and oscillations.

4 Discussion

We propose that the acrosome reaction relies upon several pathways in order to avoid entering an oscillatory state due to the IP_3 receptor dynamics. Noting that our model demonstrates high sensitivity to the parameters chosen, it is easy to imagine that without a robust ability to modulate calcium responses, an oscillatory calcium regime would be easy for the sperm head to achieve as observed in other cells with IP_3 receptors. Ordinary differential equation models such as ours can demonstrate that a wide variety of behaviors are possible, and it is likely that if a biochemical pathway is overly sensitive to reaction rates, any inherent randomness of the system would allow for different behavioral outcomes. Biological systems may exploit *multiple* pathways in order to avoid such issues, when the goal is to achieve one particular behavior with high fidelity. Reproduction is one area where high fidelity in cellular responses may be crucial for survival.

Because sperm have been shown to have similar calcium-related components as other cells, utilizing pleural calcium pathways to stabilize the calcium reaction to equilibrate at a higher calcium concentration is possible. Higher calcium concentrations are required for downstream events such as membrane fusion and acrosomal release. Evidence for pseudo-oscillatory behavior was shown in Tesarik et al. (1996), where some cells were observed to have two peaks in calcium before acrosomal exocytosis, and fully oscillatory behavior was seen in a fraction of cells in Kirkman-Brown et al. (2004). These observations may indicate that the acrosome reaction is a damped oscillatory calcium response that serves to re-equilibrate calcium to a higher resting level. Our model indicates that this could be driven, in part, by components that would create an oscillatory or spiking response observed in other cell types, but utilizing different feedback mechanisms.

To achieve a sustained elevation of calcium in the sperm head, one cellular feedback mechanism could be to exploit the inhibition of phospholipase C by a protein kinase. This would damp out otherwise oscillatory behavior. It is possible that sperm react to multiple agonists in order to stimulate two protein kinases (PKC and PKA) and their pathways using different receptors (GPCRs would activate PKA, whereas other receptors may only activate PKC). Preliminary investigations to extend this current simplified model indicate that PKA interactions with PLC and IP_3 receptors, as well

as calcium channels, would have a damping effect. However, in this work, we have chosen to focus on the PKC portion of this pathway, due to the complexities of the PKA pathway. For instance, upstream of PKA, cAMP production is affected by transmembrane voltage and pH levels that we are not including in this model. Future work to investigate this part of the signaling pathway must incorporate these effects to be realistic and would likely elucidate another potential damping mechanism for calcium oscillations.

How important the PKA pathway is compared to the PKC pathway in the acrosome reaction is an open question. Progesterone binding has been shown to be pertussis toxin insensitive, so it would not be involved in GPCR-related downstream events involving PKA. This does mean that the acrosome reaction *can* occur without the PKA part of the pathway being activated. However, the feedback from PKA on various components of the pathway may indicate that ZP proteins could stimulate a more stable biphasic response. Thus, if a non-reacted sperm reaches the zona, the ZP glycoproteins, perhaps in conjunction with progesterone or other potential agonists in the cumulus, might enforce acrosomal exocytosis instead of inducing oscillatory responses or aborted acrosome reactions. There is some evidence for this in rodent species, which are far less sensitive to progesterone and have not been observed to achieve calcium oscillations (Florman 2014).

It is important to note that the role of PKC in the acrosome reaction is controversial. The study Bonaccorsi et al. (1998) did demonstrate that the calcium increases were PKC-independent in human sperm, but the time-dependent data on calcium increases after progesterone stimulation are on the shorter timescale (about 2 min), which would just include the initial spike in calcium instead of the longer timescales necessary for the acrosome reaction to be completed. This may be evidence that the initial spike is not dependent on PKC and mostly relies upon IP_3 receptor channels, but does not rule out PKC activity stabilizing the calcium response on a longer timescale.

One other feature of our model is that calcium oscillations must be accompanied by IP_3 oscillations. Further experiments must be done to confirm whether this is the case. Mathematical models, motivated by experimental work to uncover the role of IP_3 in oscillations (Harootunian et al. 1988), have demonstrated that calcium oscillations in some cell types need not be accompanied by IP_3 oscillations (Sneyd et al. 2006). The models in Sneyd et al. (2006) involve calcium release from the endoplasmic reticulum, which can serve as a calcium store in a similar manner as the acrosome in sperm. Interestingly, Sneyd et. al. find that for pancreatic acinar cells, which have calcium responses that occur on slower timescales, IP_3 oscillations are necessary. The calcium responses observed in sperm cells are also on slower timescales compared to many other cell types, and our model does have IP_3 oscillations accompanying the calcium oscillations.

The oscillatory behavior in the model proposed here is reminiscent of calcium oscillations that occur in many other cells, which have been modeled extensively. For example, in pituitary gonadotrophs, periodic calcium release from the endoplasmic reticulum (ER), which acts as a calcium store similar to the acrosome, and biphasic calcium responses are also observed (Iida et al. 1991). Calcium models for these cell types have been proposed, and biphasic calcium responses have also been observed experimentally (Li et al. 1994, 1995). While the mathematical models for the cell types are

different and involve voltage dependence, they are able to capture both oscillations and biphasic responses. Unlike the calcium behavior in pituitary gonadotrophs, the acrosome reaction is not a periodic calcium release but rather a biphasic calcium response that occurs on a much slower timescale as part of an end-stage event for the sperm cell. However, the similarities in these cell types demonstrate that receptor-induced calcium oscillations and biphasic responses due to calcium stores are applicable to more cell types than sperm. We also refer the interested reader to Keener and Sneyd (2009) for an overview of mathematical models for calcium responses in a broad range of cells.

Other cells besides sperm also have calcium-dependent exocytotic events, such as neurotransmitter release (Augustine 2001; Becherer et al. 2003), and some models for these calcium-driven events have been proposed (for instance, see Bertram et al. 1999). While sperm are not considered excitatory cells like neurons, and the acrosome reaction is not a periodic event, sperm express similar protein complexes as other vesicle-releasing cells (Blas et al. 2005) and there is evidence that analogs of neurotransmitter receptors are involved the acrosome reaction (Hiradate et al. 2014). We have not focused on specific receptor dynamics in this first model, but rather assumed basic receptor on/off model in order to focus on downstream events known to be involved in the acrosome reaction.

Because our model is a first attempt at understanding the complex interactions of this pathway, we chose to emphasize simplicity and focus on just a few of the fundamental aspects of the calcium cascade in the acrosome reaction. There are many other important aspects of this reaction which we have omitted in our model which will be the subject of further studies. For instance, spatial effects and the potential for direct stimulation of calcium transporters by the receptor should be examined. We also have not modeled the effects of voltage dependence of calcium channels nor lipid involvement in such channels in our model, though there is experimental evidence for this (for instance, see Cohen et al. 2014). Another defining attribute of the acrosome reaction is a sustained increase in pH, which we also have not included in our model.

While all of these features may play an important role in the calcium response and the acrosome reaction in general, our simplified model does capture the qualitative behaviors of calcium responses measured experimentally. We regard this model as a starting point to investigate the calcium response of the acrosome reaction. As noted in Sánchez-Cárdenas et al. (2014), oscillations in calcium prevent premature acrosome reaction and exocytosis. While many secondary messengers have been shown to play a role in this reaction cascade, our model demonstrates the potential of secondary messengers to stabilize the system in a high calcium state. We believe this stabilization—through exploitation of multiple biochemical pathways and perhaps multiple agonists like ZP and progesterone—may serve to promote acrosomal exocytosis at the correct stage for successful fertilization of the oocyte.

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