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REVIEW ARTICLE

Reviewing mathematical models of sperm signaling networks

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Abstract

Dave Garbers' work significantly contributed to our understanding of sperm's regulated motility, capacitation, and the acrosome reaction. These key sperm functions involve complex multistep signaling pathways engaging numerous finely orchestrated elements. Despite significant progress, many parameters and interactions among these elements remain elusive. Mathematical modeling emerges as a potent tool to study sperm physiology, providing a framework to integrate experimental results and capture functional dynamics considering biochemical, biophysical, and cellular elements. Depending on research objectives, different modeling strategies, broadly categorized into continuous and discrete approaches, reveal valuable insights into cell function. These models allow the exploration of hypotheses regarding molecules, conditions, and pathways, whenever they become challenging to evaluate experimentally. This review presents an overview of current theoretical and experimental efforts to understand sperm motility regulation, capacitation, and the acrosome reaction. We discuss the strengths and weaknesses of different modeling strategies and highlight key findings and unresolved questions. Notable discoveries include the importance of specific ion channels, the role of intracellular molecular heterogeneity in capacitation and the acrosome reaction, and the impact of pH changes on acrosomal exocytosis. Ultimately, this review underscores the crucial importance of mathematical frameworks in advancing our understanding of sperm physiology and guiding future experimental investigations.

KEYWORDS

dynamical modeling of sperm physiology, signaling regulatory networks, sperm capacitation, sperm intracellular and acrosomal pH, sperm ion channel regulation

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INTRODUCTION

In the context of this special issue in tribute to Dave Garbers, it is worth remarking that his endeavor to understand the sperm-egg dialog resulted in fundamental contributions to establish the pathways that regulate sperm swimming that is key for reproduction. His discovery of the first sperm chemoattractant peptide from sea urchin eggs and the cloning of its receptor (membrane guanylyl cyclase), established the first cell-surface receptor that could directly generate a low-molecular-weight second messenger, cGMP. Thereafter, he identified and cloned the first subunit of a sperm-specific Ca^{2+} channel, simultaneously with the Clapham group (Kirichok et al., 2006; Ren et al., 2001), now named CatSper, that turned out to be an essential element of sperm physiology in many species (Garbers et al., 2006).

Garber's findings began to unveil and unravel the complexities of sperm chemotaxis, hyperactivation, capacitation and the acrosome reaction (AR). As mathematical modeling is a systematic and quantitative approach that enhances our understanding of how cells operate, enabling predictions, and guiding research, we decided to tackle these fascinating and important signaling networks using this powerful strategy.

Mathematical modeling is valuable for studying cell function and foretelling behavior because it enables scientists to simplify their intricate systems with numerous components and interactions. Models serve as a platform for testing hypotheses about cell behavior in a controlled and quantifiable manner. Investigators can manipulate variables and simulate scenarios to predict outcomes, which can then be experimentally verified. Mathematical models can forecast how cells will respond under different conditions or stimuli, aiding in experimental design and targeted interventions. Modeling allows researchers to explore a wide range of scenarios, some of which are experimentally unavailable or extremely time-consuming, leading to more efficient research strategies.

Furthermore, data integration can be performed by using models to provide a comprehensive view of cellular function. These possibilities can reveal hidden relationships and mechanisms governing cell behavior, uncovering novel biological insights. Since mathematical models allow testing compounds on cells without doing experiments, they are a powerful tool for identifying key steps in cell signaling and developing potential therapeutic strategies. Altogether, the gathered information can deepen our understanding of cell function, disease mechanisms and drug targets. There are several excellent general reviews on various aspects of modeling cell signaling (Azeloglu & Iyengar, 2015; Janes & Lauffenburger, 2013; B. Kholodenko et al., 2012; Klipp & Liebermeister, 2006; Loos & Hasenauer, 2019; Morris et al., 2010).

Finally, it is worth not forgetting that mathematical modeling can help to assimilate complex biological concepts, making them more accessible for students and facilitating communication among scientists from different fields.

In this review, we will focus on efforts conducted to utilize modeling to better understand sperm physiology. Preference will be given to modeling attempts to study sperm signaling pathways such as chemotaxis in the sea urchin, capacitation in the mouse and the AR in the human. A summary of other modeling efforts in sperm will also be discussed.

We present modeling within a systems biology approach, which provides an integrated global behavior of many component interacting systems. We focus on dynamical and functional aspects of regulatory networks. The structural and functional properties were obtained from studies found in the scientific literature and information gathered from experiments. The formalisms we employ may be deterministic or stochastic in terms of discrete or continuous variables, dependent on discrete or continuous time. For a given process, any combination of the above may be more appropriate. In what follows, we show (a) deterministic three-state discrete time logic networks and systems of coupled differential equations for invertebrate swimming regulation and chemotaxis; and (b) hybrid (deterministic/probabilistic) models for mouse sperm capacitation and deterministic two-state discrete time Boolean models for the human sperm acrosome reaction. An important aspect of models with a discrete-time evolution is whether synchronous or asynchronous updating is considered. Additional discussion of the above variants, which we implement further on, can be found in the mathematical modeling box. From our point of view, the choice of formalism is dictated by the aspects of the process under study and the issues under investigation. Limitations and advantages of the different approaches are addressed later on

DISCRETE MODEL FOR MARINE INVERTEBRATE Ca^{2+} SIGNALING PATHWAY

While navigating towards the egg, sperm from sea urchins *Strongylocentrotus purpuratus* and *Lytechinus pictus* respond to speract, a decapeptide found in the external layer of eggs that diffuses in the sea upon spawning (Hansbrough & Garbers, 1981). Speract binding to its receptor on the sperm flagellar membrane triggers a signaling cascade intricately regulated by membrane permeability changes to specific ions. This cascade, known as the speract-activated signaling pathway (SASP), induces a series of cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) oscillations. These fluctuations in $[Ca^{2+}]_i$ are closely tied to alterations in sperm swimming behavior (Darszon et al., 2008; Wood et al., 2003, 2005, 2007). It is worth noting that parallel efforts have been devoted to *Arbacia punctulata* and resact, its chemoattractant (reviewed in Kaupp et al., 2008).

In a two-dimensional experimental setting, sea urchin sperm exhibit circular swimming patterns near the surfaces (Cosson et al., 2002). As the flagellum detects an increase in the rate of Ca^{2+} influx, its beating is altered, leading the spermatozoon to execute distinct turns followed by straighter swimming motions (Böhmer et al., 2005; Guerrero et al., 2010; Wood et al., 2005, 2007).

The characterization of all elements involved in the speract-activated signaling pathway is essential for deciphering the intricacies of sperm behavior to reach the egg and fertilize it. The insights that

arise from modeling efforts broaden our understanding of cellular communication and regulation.

Given the inherent uncertainty surrounding the values of numerous reaction rates, concentrations, and the potential presence of various elements within the pathway, we opted for an initial discrete modeling approach for the SASP. This method carefully considers the known elements within the pathway (Espinal et al., 2011).

The pathway

The signaling pathway for Ca^{2+} oscillations in *S. purpuratus* sperm flagellum can be divided into three main components:

1. Triggering system: Comprising the speract-receptor complex (SR), guanylate cyclase (GC) linked to SR (Hansbrough & Garbers, 1981), cyclic guanosine monophosphate (cGMP) production through GC activation (Álvarez et al., 2014; Garbers, 1976; Kaupp et al., 2008; Nishigaki et al., 2004), and the cGMP-dependent K^+ channel (KCNG), which opens upon cGMP binding, leading to K^+ efflux and the concomitant hyperpolarization of the membrane potential (V_m) (Babcock et al., 1992; Bönigk et al., 2009; Cook & Babcock, 1993b; Galindo et al., 2007; Strünker et al., 2006).
2. Initial Ca^{2+} influx: This phase involves several components activated by membrane hyperpolarization, including the: Na^+/Ca^{2+} exchanger (NCE) (Nishigaki et al., 2004; Rodríguez & Darszon, 2003; Su & Vacquier, 2002), Na^+/H^+ exchanger (NHE) discovered by the Prof. Garber's group (Lee & Garbers, 1986; Wang et al., 2003), a hyperpolarization-activated and cyclic nucleotide-gated channel (HCN) (Galindo et al., 2005; Gauss et al., 1998). At the time Espinal et al.'s model was published (2011), the presence of CatSper —a sperm-exclusive Ca^{2+} channel— was unknown, and inactivation removal of high and low voltage-activated Ca^{2+} channels (HVA and LVA) were considered important (Cook & Babcock, 1993a; Espinal et al., 2011; Nishigaki et al., 2004), as well as the dynamics of cyclic adenosine monophosphate (cAMP) concentrations (Kaupp et al., 2003, 2008).
3. Calcium oscillation system: This phase, triggered by pH_i elevation (Lee & Garbers, 1986; Wang et al., 2003), was thought to involve the cAMP-dependent Ca^{2+} channel (cAMPCC) (Cook & Babcock, 1993a; Nishigaki et al., 2004), the opening of HVA and LVA channels (Darszon et al., 2011; Wood et al., 2005), and the involvement of Ca^{2+}-dependent Cl^- channel (CaCC), Ca^{2+}-dependent K^+ channel (CaKC) (Espinal et al., 2011; Espinal-Enríquez et al., 2014). Further on, the presence and involvement of the CatSper channel (Espinal-Enríquez et al., 2017), which generates Ca^{2+} increases and peaks, was considered.

Furthermore, constant passive Ca^{2+} extrusion mechanisms, such as Ca^{2+} pumps (CaP) (Okunade et al., 2004), and NCE (Nishigaki et al., 2004; Rodríguez & Darszon, 2003; Su & Vacquier, 2002), would maintain baseline Ca^{2+} levels. These mechanisms are cyclically repeated to generate a sequence of $[Ca^{2+}]_i$ oscillations and sperm flagellar movement. Figure 1a shows the signaling pathway activated by speract in the sea urchin sperm flagellum.

The model

To simulate speract-induced dynamics in the sperm flagellum, we employed a discrete model based on genetic regulatory network principles (Kauffman, 1969). This logical network extends the traditional Boolean networks since it uses discrete variables with three states (0 for off, 1 for on, and 2 for intermediate) to represent four network elements, namely V_m, voltage-dependent Ca^{2+} channels (Cavs HVA and LVA), and the Ca^{2+} ion (Espinal et al., 2011).

This approach enabled us to capture integrated complex dynamics in the sperm flagellum regulated by speract. In what follows, we provide a general description of the discrete model for the Ca^{2+} oscillations triggered by speract (Figure 1b).

The state of each node, σ_n, is influenced by its set of regulators, denoted by $\sigma_{n1}, \sigma_{n2},..., \sigma_{nk}$. At each discrete time step, the value of σ_n is determined by a regulatory function, F_n, which takes into account whether its regulators activate or inhibit it. Each node possesses its distinct regulatory function, which is crafted based on specific regulatory interactions. Developing these functions can be a complex process, often necessitating certain assumptions due to the limited information available. As an example, we have provided an illustrative regulatory function for the node corresponding to cGMP in Box 1, where variables are denoted by x instead of σ. These regulatory tables can grow more intricate, as is the case of the 432-entry table for Ca^{2+}, involving seven regulatory nodes, three of which have three states. We devised these regulatory functions from biological knowledge, particularly electrophysiological data, gleaned from existing literature and our own research. A detailed description of the discrete formulation to simulate a signaling pathway is presented in Box 1.

The network's evolution commences from an initial state $\{\sigma_1(0), \sigma_2(0), ..., \sigma_n(0)\}$ at $t = 0$, progressing through intermediate states until it converges into a recurring pattern termed an "attractor." The basin of attraction encompasses all initial states that lead to a particular attractor. Within the same network, multiple attractors may exist, each with its associated basin. In our context, these attractors represent stable Ca^{2+} oscillations that govern the repositioning of sperm via Ca^{2+}-induced changes in flagellar curvature. The period of the attractor is the duration between consecutive Ca^{2+} peaks (Figure 1c).

It's worth noting that this formalism assumes that network nodes are updated synchronously. Ongoing efforts are being made to assess the limitations of this assumption by considering a semicontinuous model that incorporates asynchrony through characteristic time scales for each node.

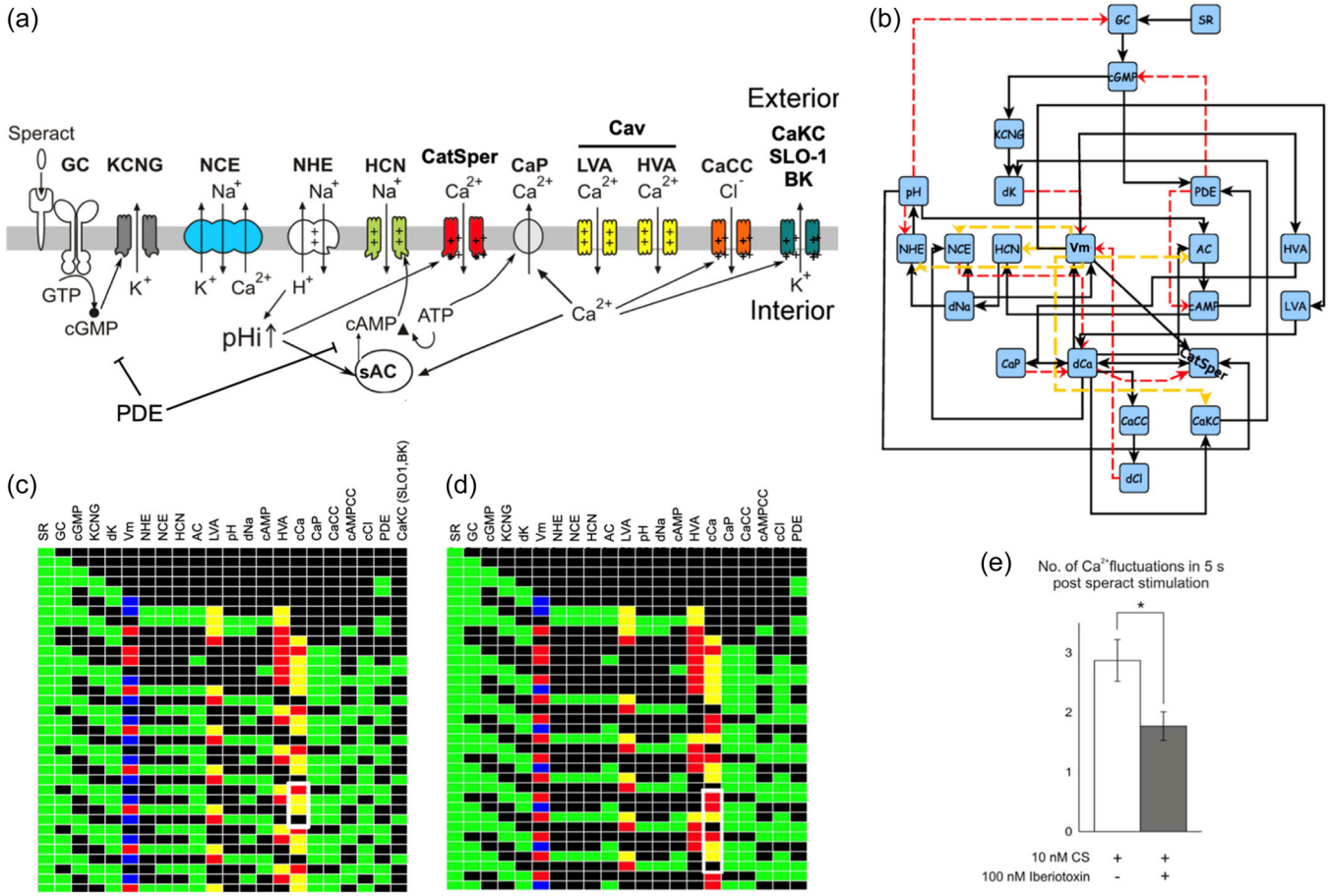


FIGURE 1 Discrete model for the speract-activated Ca^{2+} signaling pathway in *S. purpuratus* sperm flagellum. (a) Illustration of all the components involved in speract signaling and featured in the model. The ++ in the channels indicates voltage dependence. (b) Logical regulatory network model represented as a wiring diagram in which the components are nodes, and the interactions correspond to edges. The black and red edges represent activating and inhibitory interactions, whereas the yellow edges represent variable effects of membrane potential (V_m) on its target nodes. (c) Network dynamics as a sequence of states (rows) of the indicated nodes in the presence of speract (node 0). The colors represent the dynamical state of the nodes. In the majority of them, black indicates inactivation and green activation. For nodes 10 and 14 representing HVA and LVA Ca^{2+} channels, respectively; inactive states are denoted by black squares, closed states by yellow, and open states by red. Regarding V_m (node 5), a resting potential is represented by black squares, hyperpolarization by blue, and depolarization by red. In the case of the $[Ca^{2+}]_i$ node (dCa) (node 15), yellow indicates tonic elevation, red signifies a supratonic increment, and black denotes the basal state. After a transient, a periodic attractor is reached as the repeated pattern indicates. The dCa-oscillation period is highlighted with a white rectangle. (d) Impact of removal of the node representing CaKC (SLO-1 or BK) on the dynamics and attractor periodicity; (e) Experimental validation of the increase in the period of speract-induced dCa-oscillations by CaKC removal using the pharmacological inhibitor iberiotoxin.

Model predictions

Our initial logical network effectively replicated various experimental findings and generated predictions that were subsequently validated through experiments. Specifically, we observed that creating a simulated blockade of CaKC resulted in an elongation of the time gap between successive Ca^{2+} peaks as well as an increase in the average $[Ca^{2+}]_i$ (see Figure 1d). When we conducted experiments with Iberiotoxin, a specific blocker of the CaKC channel, we noted, in agreement with the model prediction, that the time interval between Ca^{2+} peaks in speract-dependent $[Ca^{2+}]_i$ oscillations increased (Figure 1e). Additionally, the average $[Ca^{2+}]_i$ increased in this experimental condition (Espinal et al., 2011).

Later on, we explored the effect of a multitarget drug called niflumic acid (NFA) on the SASP using our model (Espinal-Enríquez et al., 2014), taking into account its influence on the permeability of CaCC, HCN, and CaKC. While NFA affects these three channels differentially in the μM range (Cheng & Sanguinetti, 2009; Greenwood & Large, 1995; Guerrero et al., 2013), two scenarios emerged from the ongoing experimental debate concerning whether NFA's effect on CaKC is inhibitory or activating. Given the mounting evidence suggesting NFA-dependent activation of CaKCs, our model necessitated the inclusion of more NFA-sensitive ionic channels in the SASP to reconcile the experimental results reported by Wood et al. (2007). In that publication, we proposed that CatSper could potentially fulfill the role of such a channel.

BOX 1 Mathematical modeling of regulatory networks

The mathematical description of the molecular functioning of living organisms is often carried out using complex regulatory networks. With the development of high-throughput experimental techniques during the last two decades, it is possible to know the interactions between hundreds or thousands of elements within a cell, whether genes, proteins, metabolites, ions, or all of them. These interacting elements are identified as the nodes of a network, which is then represented by a set of N connected variables $\{x_1, x_2, \cdots, x_N\}$, each one containing the relevant information of a particular type of element in the system. For instance, in gene regulation networks, the variable x_n represents the state of expression of the n^{th} gene: either the gene is expressed, or it is not. In such a case, each variable x_n acquires only two values, 0 and 1. In protein, metabolic, and signaling networks, one is often more interested in the *concentration* or *continuous activity* of the elements (proteins, ions, metabolites, electric currents, etc.). Therefore, in the case where hundreds of copies of the same molecule or ion can be present in the system, the variable x_n represents the concentration of the n^{th} element and acquires a continuous value between 0 and a maximum $C_n^{\max}$ (this maximum value can always be rescaled to 1). In other systems, a given element can be present in several copies but not so many as to consider it as a continuous concentration. Moreover, the number of copies of this element can randomly fluctuate throughout time. In this situation, the corresponding variable x_n is typically stochastic and acquires integer values ranging from 0 to a maximum integer $M_n^{\max}$.

The dynamical behavior of the system under consideration for each of the cases mentioned above is described by different mathematical formalisms. First, one must construct the network, namely, determine which node (or element) interacts with which other ones. This is done by assigning to each variable x_n a set of k_n "regulators" $\{x_{n_1}, x_{n_2}, \cdots, x_{n_{k_n}}\}$:

$$\{x_{n_1}, x_{n_2}, \cdots, \quad x_{n_{k_n}}\} \rightarrow x_n. \tag{1}$$

These regulators are the elements that interact with (or produce) x_n and will determine its value throughout time. The network is fully constructed when each node has been provided with a set of regulators. For networks of real organisms, this assignment is very crafty and can take years of work, either doing experiments or reading the relevant literature (or both). Then, the second (and perhaps more interesting) part of the mathematical modeling of the network comes into play: What type of interactions are going to be considered, and how to implement them mathematically? Here, we review only two different approaches: discrete and continuous.

Discrete approach

To our knowledge, the first discrete mathematical model of a biological system is the one proposed by Warren McCulloch and Walter Pitts in 1943 to describe the dynamics of neurons and neural networks in the brain (McCulloch & Pitts, 1943). In the McCulloch-Pitts model, the activation state of a neuron can have only two states: active (+1) or inactive (-1). Although this is clearly a simplification of the membrane potential dynamics that make a neuron fire, this discrete model has shed insights into memory and pattern recognition processes, paving the way to all the wonders of deep learning and artificial intelligence we are familiar with nowadays. Almost 30 years later, Stuart Kauffman in 1969 (Kauffman, 1969) and René Thomás in 1973 (Thomás, 1973) proposed discrete models to describe the dynamics of gene transcription networks, as genes can be only in two states: either the gene is expressed, or it is not. This Boolean approach has been corroborated in recent single-cell experiments able to measure the expression of one given gene, as it has experimentally been observed that gene expression is a binary phenomenon and occurs in discontinuous "bursts" (Lin & Galla, 2016; Sanchez & Golding, 2013). Therefore, in modeling gene expression dynamics, each gene is represented by a Boolean variable x_n that takes two values, 0 (the gene is not expressed) and 1 (the gene is expressed).

For metabolic and signaling networks, the discrete approach can also be used as an approximation that only considers the presence or absence of certain elements (ions, proteins, or metabolites), providing a coarse-grained description of the system's dynamics. Sometimes, what is important is the conformation of a protein (e.g., it is phosphorylated, or it is not), while some other times, the continuous character of the variables must be discretized. When applying the discrete approach to a continuous system, some thresholds must be established to determine when the concentration of a given element x_n is low enough to consider it as absent (0), or when this concentration is high enough to consider this element as present (1). The discrete approximation of a continuous system is useful for describing the network dynamics by knowing only the logical relationships between its nodes, but it is useless for predicting specific concentrations or fluctuations around mean values.

In the Boolean approach, once each node has been provided with a set of regulators as in Equation (1), the network dynamics are obtained by assigning to each node x_n a Boolean function F_n that depends on its regulators such that:

$$x_n(t + \tau) = F_n(x_{n_1}(t), x_{n_2}(t), \cdots, x_{n_{kn}}(t)). \quad (2)$$

In the above equation, τ is the average time it takes a node to respond to changes in the expression of its regulators, and F_n is a Boolean function that depends on the state of these regulators at a given time point. Note that not only the value of the nodes is discrete, but also the time which increases in discrete steps of length τ. The following example illustrates a typical Boolean function depending on three variables.

All possible configurations of the regulators			Output of the Boolean function
x_{n_1} = *GC*	x_{n_2} = *PDE*	x_{n_3} = *cGMP*	x_n = *cGMP* = $F_n(x_{n_1}, x_{n_2}, x_{n_3})$
0	0	0	**0**
0	0	1	**1**
0	1	0	**0**
0	1	1	**0**
1	0	0	**1**
1	0	1	**1**
1	1	0	**0**
1	1	1	**0**

The state of cyclic guanosine monophosphate (cGMP) depends on the presence or absence of three other regulators: guanylate cyclase (GC), a phosphodiesterase (PDE), and itself (Espinal et al., 2011). Note that PDE is a dominant repressor, as cGMP is absent every time PDE is present. This is only one node of the network that regulates calcium dynamics in the sea urchin sperm. Constructing this type of function for all nodes of a real network can take years of work.

When constructing this type of truth tables, one faces the difficult problem that regulation is a combinatorial process. Some people carelessly talk about the inhibitory or activating nature of *individual* regulators when it is the *combination* of all the regulators that determines the state of the regulated node. For instance, in the table that appears in this Box, GC activates cGMP while PDE inhibits it. What happens when the activating and inhibitory regulators are both present? This type of conflict always appears and must be resolved through experiments, reading the literature or by guessing (and corroborating the results *a posteriori*). In the case of cGMP, the repressor PDE is dominant.

There are systems for which it is necessary to consider nodes with more than two discrete states. For instance, the immune system's inflammatory response depends on whether specific cytokines are present in low, medium, or high levels. In such cases, the discrete values of the corresponding variable x_n can be 0, 1, and 2, and its associated discrete function F_n will output one of these three values depending on the configuration of the regulators. Analogously, the membrane potential of a neuron or a sperm cell is best characterized by three values: hyperpolarized (0), resting (1), and depolarized (2). In the context of the sea urchin fertilization regulatory network, $[Ca^{2+}]_i$ also requires three values (Espinal et al., 2011). However, the essence of the discrete approach does not change. If there are too many levels of discretization, it is better to use a continuous approach.

Continuous approach

Since the pioneering work by François Jacob and Jacques Monod in 1961 to explain gene expression control in bacteria to utilize lactose as a source of food, namely the *lac-operon* model (Jacob & Monod, 1961; Lewis, 2011), continuous models based on ordinary differential equations (ODEs) have been widely utilized to describe the dynamics of metabolic and signaling networks. Before them, Alfred J. Lotka and Vito Volterra also formulated continuous models to describe the dynamics of competing species in ecosystems, while Alan L. Hodgkin and Andrew Huxley used the continuous approach to describe the ionic mechanisms governing the propagation of electric currents in the neurons of giant squids (Murray, 2002). This type of models has historically been considered more "realistic" than discrete models, as the continuous approach allows us to compute not only the average value of the elements' concentrations, but also fluctuations around these averages and their temporal organization, which is unviable within the discrete approach. However, one must be aware that the continuous approach is also an approximation, as it tacitly assumes the *well-mixed hypothesis*, namely that all elements in the system are well-mixed in a homogeneous medium. While some systems conform to this hypothesis (like a chemical reactor or a chemostat), some others do not (like a Petri dish, a cell or a tissue).

In the continuous approach, once each element x_n has been provided with a set of regulators as in Equation (1), the rate of change of its activity or concentration can be mathematically written as

$$\frac{dx_n}{dt} = \sigma_n - \delta_n + H_n(x_n, x_{n_1}, x_{n_2}, \cdots, x_{n_{kn}}). \quad (3)$$

This is a typical ODE that determines the temporal evolution of x_n when the network element interacts with (e.g., reacts with, receives signals from, is modified by) the set of its k_n regulators. In the above equation, σ_n represents a source of production rate constant that is independent of the regulators considered as variables in the model; δ_n is a first-order decay, degradation, or removal rate constant.

The function $H_n(\cdot)$ governing the interaction-dependent dynamics is typically a composite nonlinear function of the concentrations of the element x_n and its regulators $(x_{n_1}, x_{n_2}, \cdots, x_{n_{k_n}})$. In one of the simpler scenarios, $H_n(\cdot)$ is a sum of positive and negative fluxes according to mass action kinetics. More often, $H_n(\cdot)$ is a sum of case-specific nonlinear functions of subsets of regulators. Each of these nonlinear functions calls into action a subset of specific parameters. To illustrate this, consider the ODE describing the dynamics of the cGMP concentration proposed by Priego-Espinosa et al. (2020), rewritten here as:

$$\frac{dG}{dt} = \sigma - \delta G + \underbrace{\theta(k_H R_H + k_L R_L)}_{H(\cdot)} \quad (4)$$

In this equation, where G denotes the cGMP concentration, the function $H(\cdot)$ represents the production of cGMP by its regulators which are two forms of the SAP-receptors with guanylate-cyclase activity. The function is a simple sum of the concentrations of the high and low activity receptors, respectively R_H and R_L, multiplied by the respective rate constants, k_H and k_L, and a scaling constant θ. In this formulation, the changes in cGMP concentration are determined by five parameters, σ, δ, θ, k_H and k_L. The active receptor concentrations R_H and R_L are themselves variables governed by specific differential equations, involving their specific parameters. In a system of ODEs describing a moderately simple biological system, the number of parameters can rapidly get out of hand.

The quantitative information on the functions $H(\cdot)$ and associated parameters is not always available and sometimes confusing, as some of the kinetic constants are measured under some experimental conditions while others are measured under quite different experimental conditions. Furthermore, very often a whole set of constants is unknown. Nonetheless, when molecular abundances and fluctuations are at the focus of the research, specifying the values of all those kinetic parameters is mandatory, which can be achieved only for small networks (or circuits), such as the lac operon, the lysis-lysogeny switch of the lambda bacteriophage, and the Hodgkin-Huxley system. However, for networks such as the one considered in this work, only a small set of the kinetic parameters can be estimated from quantitative observations or constrained by literature. Some assumptions about the values of the remaining parameters must be made to reach relevant conclusions, which demands an examination of the robustness of the results to changes in parameter values. One overarching assumption that often remains implicit is that, in addition to the well-mixed hypothesis mentioned above, the important parameters do not change regardless of the experimental (boundary) conditions. This is a strong hypothesis that often remains to be assessed.

Hybrid models

There are systems in which some of the variables are best described by discrete values whereas some others are best described by continuous values. This is the case, for instance, when metabolites can activate or inhibit the expression of a specific gene. While metabolites can be represented by continuous variables, gene expression is well described as a binary process. For such systems, hybrid models must be formulated, as some variables change continuously while others change in discrete steps. Other types of hybrid models are also possible, such as deterministic/stochastic, synchronous/asynchronous, and combinations of all of them. We do not consider discrete/continuous hybrid models in this work, but the interested reader can find useful information in the work by Albert (2007). However, a deterministic/stochastic model is presented for the capacitation study where, in the otherwise deterministic model, ion-concentrating nodes are linked to input nodes following a neural network (McCulloch-Pitts) probabilistic formalism. This avoided the need for the consideration of asynchronous updating (Aguado-García et al., 2021).

The aforementioned studies addressed, at the time, the significance of $[Ca^{2+}]_i$ fluctuations in sea urchin swimming and the specific identification of Ca^{2+} channels in the flagellum responsible for these changes. While research had pointed to the involvement of the CatSper channel in sperm chemotaxis (Seifert et al., 2014), the extent of its contribution to the signaling pathway remained uncertain, leaving room for questions about the involvement of other Ca^{2+} channels like LVA and HVA.

To address the controversy pointed out above, we explored three model variants dependent on the Ca^{2+} channels considered: Model-I with HVA and LVA, Model-II with CatSper, LVA, and HVA, and Model-III with only CatSper. The experimental comparison shown in Table 1 suggested that CatSper was indispensable and likely the primary Ca^{2+} channel.

As for other Ca^{2+} channels, removing Cavs from the network altered the Ca^{2+} dynamics without significantly affecting the average Ca^{2+} concentration and elevation rate. This suggests that these channels may play a role in fine-tuning sperm movements during processes like chemotaxis and chemokinesis. However, proteomic efforts have not detected LVA or HVA in sea urchin sperm flagella to date (Espinal-Enríquez et al., 2017; Seifert et al., 2014; Trötschel et al., 2019).

These findings prompted us to design pharmacological protocols to investigate whether CatSper contributes to the $[Ca^{2+}]_i$ response induced by speract in *S. purpuratus* sperm. We confirmed the presence of all CatSper subunits except CatSper3 through proteomics. Additionally, the nonspecific CatSper channel blockers, Mibefradil

TABLE 1 Comparative model-experiment study of three network models

Treatment	Measurement	Model-I LVA, HVA	Model-II LVA, HVA, CatSper	Model-III CatSper	Experiment
pH blockage	Ca level	–	–	↘	↘
PDE blockage	Ca level	–	–	↘	↘
CaKC blockage	Ca level	↗	↗	↘	↘
	Period	↗	↘	↗	↗
NFA addition	Ca level	–	↗	↗	↗
	Peak	↘	↗	↗	↗
	Amplitude	↘	↘	↗	↗
	Period	↘	↘	↗	↗
	Success ratio	0.25	0.375	1	

Note. The table presents a comparative analysis of three network models through experimentation. The models differ in the choice of Ca2+ channels indicated in the Table. The first column indicates the pharmacological treatment under investigation, while the second column specifies the measured quantity: in experiments, Ca2+ level refers to the Ca2+ oscillation steady-state average concentration; for the models, it is the same steady-state Ca2+ concentration in population of the oscillations taken from the average over 100,000 simulations over randomly chosen initial conditions. Peak is the top Ca2+ oscillation value; amplitude, the highest increase in the Ca2+ oscillations; and period, the steady state oscillation period. Columns 3, 4, and 5 present results from Model-I, Model-II, and Model-III, respectively. Column 6 indicates experimental determinations. ↗ denotes an increase in the indicated Ca2+ property, ↘ signifies a decrease, and "–" denotes minor changes. The success ratio is calculated as the ratio between the number of coincidences between the model and experimental results and the total type of experimental determinations. A success ratio of 1 signifies complete agreement between experimental findings and the specified model results.

and NNC-055-396 (NNC) significantly reduced the increase in $[Ca^{2+}]_i$ induced by speract, providing further support for the idea that CatSper plays a significant role in this response (Espinal-Enríquez et al., 2017). Similar results were observed in *A. punctulata* sperm stimulated by resact (Seifert et al., 2014).

Further advantages of our discrete extended Boolean model include the possibility of conducting simulations to explore in general the impact of adding or removing elements from the signaling pathway on the dynamics of Ca^{2+} oscillations, specifically focusing on their frequency and magnitude.

This approach led us to pinpoint the significance of certain elements within the pathway, including the CaKC, which is sensitive to iberiotoxin, and the CaCC, probably to TMEM16 (Loyo-Celis et al., 2021). We also discovered the effects and some operation characteristics of multitarget drugs, such as niflumic acid, which had varying impacts on their respective targets.

In addition to Ca^{2+} oscillations, our research also revealed that the signaling network operates in a critical dynamic regime, optimizing information transmission over time in evolutionary terms. This state's permanence was crucial in distinguishing indispensable elements for maintaining Ca^{2+} oscillations (see Box 2).

Some of the outcomes of the implementation of a logic discrete network for the Ca^{2+} signaling pathway are:

1. A discrete logical model can be used to discover new elements in the signaling pathway, based on the periodic behavior of a key node, in this case, $[Ca^{2+}]$. It provides a coarse-grained integrated framework for collective global behaviors.
2. With the discrete model, we could observe the effect of different drugs (even with multiple targets) on a given signaling pathway. The discrete model can be easily refined, as demonstrated by the addition of the CatSper channel, whose incorporation into the network allowed the recovery of all the aforementioned effects, and also showed that it is key for $[Ca^{2+}]_i$ oscillation maintenance.
3. The *S. purpuratus* signaling pathway system is placed in the critical regime, a dynamical property that allows the system to be resilient under perturbation and evolve at the same time, depending on the conditions. Later sections will better explain this property (see Box 1).

Despite its multiple advantages in terms of fast implementation and in-silico knockout, Boolean modeling has limitations. It simplifies the continuous nature of biological processes but does not account for detailed quantitative changes. The absence of real time and the lack of a systematic scheme for the construction of the dynamics regulatory tables are other hurdles. Additionally, the limited specificity of inhibitors, the absence of reaction rates for some nodes and the impossibility of determining them, or the need for asynchronous updating of variables, are also points to consider when this kind of model is used. In cases where precise quantitative predictions are needed, ODE models are often more appropriate. The choice between discrete and continuous modeling depends on the specific research goals and the available data and expertise. In practice, a combination of modeling approaches may be used to gain a comprehensive understanding of complex biological systems.

BOX 2 Different dynamical phases: The importance of criticality

Regulatory networks, as many dynamical systems, can operate in three different phases: ordered, critical and chaotic, depending on the values of their parameters. These phases are mainly characterized by how the system responds to perturbations. A system operating in the ordered phase would not change its behavior when a perturbation is applied. For instance, in a gene regulatory network, when a given gene is forced to be silent, all the other genes in the network will not change their pattern of expression, not even the ones that are regulated by the silenced gene. The system is essentially insensitive to perturbations. One then says that the dynamics is *frozen*. By contrast, in the chaotic phase, a perturbation applied to only one node of the network will change the behavior of a relatively large fraction of other nodes. In this case, knocking out one gene of the genetic network would change the pattern of expression of many other genes. In the chaotic phase, the network randomly propagates a small perturbation to a relatively large part of the system.

In general, neither ordered nor chaotic systems are good candidates to model biological organisms. Ordered systems do not respond to perturbations, while chaotic systems over-respond to perturbations. However, there is an intermediate point between order and chaos at which the system is not extremely sensitive to perturbations, but it is not frozen either. This is the critical point at which the transition between order and chaos occurs. In his pioneering work, Kauffman found this order-chaos phase transition in models of gene regulation networks and put forward the hypothesis that living organisms should operate at, or near, the critical phase (1969). This is more than just a reasonable thought. In many studies carried out during the 50 years after Kauffman's original work, it has been argued that there is no escape to this conclusion. If living organisms were ordered, evolution would be impossible as they would not respond to perturbations in the environment. Likewise, if living organisms were chaotic, even a small perturbation would abruptly change the organism's genetic pattern of expression, preventing it from gradually adapting to changing environments. Only systems operating at (or near) the critical point would be both robust and evolvable, two characteristics necessary to contend with new environmental challenges (M. Aldana et al., 2007). This proposition is known now as the *life at the edge of chaos* hypothesis, and it has been confirmed by several computational and experimental studies not only for gene regulation networks (Balleza et al., 2008), but also for the brain (Plenz et al., 2021), signaling networks (Espinal et al., 2011), ecosystems (Ramírez-Carrillo et al., 2018), and flocks of birds (Bialek et al., 2014; Cavagna et al., 2018), to mention just a few examples.

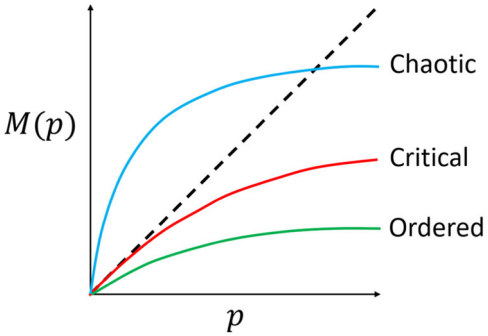
Undoubtedly, one of the most important characteristics of complex systems operating at or near the critical point is that all their parts are strongly correlated. Therefore, most likely the system will respond collectively to perturbations acting on any of its parts. This collective response is not random (as in the chaotic phase) but determined by the correlations that are created between the different parts of the system. In the ordered phase, the size of the system's response (i.e., the number of parts that change their behavior after a perturbation) is always very small, while in the chaotic phase, it is always random and large. At the critical point the size of the response is characterized by a power-law distribution of the form $P(s) \approx Cs^{-\alpha}$, where C is a constant, s is the size of the response, and α is a positive number. This power law implies that perturbations of any scale can generate small, medium, or large responses. The importance of criticality is that systems operating at the critical point have the capability of mounting collective responses of appropriate size to contend with external perturbations generated by changing environments. For more information about critical systems in biology and other areas, see Mora and Bialek (2011), and Roli et al. (2017).

How to measure criticality?

Let us assume that at time $t = 0$ a small fraction p_0 of the nodes of the network is perturbed by changing their values. Since these perturbed nodes regulate downstream the values of other nodes, the initial perturbation can propagate to other nodes in the network producing a new fraction p_1 of perturbed nodes at the next time step $t = 1$. The process continues until the size of the perturbation reaches a steady value p_∞:

$p_0 \rightarrow p_1 \rightarrow p_2 \rightarrow p_3 \rightarrow \cdots \rightarrow p_\infty$

The size p_t of the perturbation at time t clearly depends on the size p_{t-1} of the perturbation at the previous time step: $p_t = M(p_{t-1})$.



In the previous equation, the mapping $M(p)$ is a function that determines how the perturbation propagates through time. The above figure shows the characteristic curves of $M(p)$ that correspond to the three different dynamical phases: ordered, critical and chaotic. It can be shown that the slope at the origin of $M(p)$, namely,

$S = \left[\frac{dM(p)}{dp}\right]_{p=0}$ is the most important parameter that determines the dynamical phase, such that:

$S < 1 \rightarrow$ *Order* $S = 1 \rightarrow$ *Critical* $S > 1 \rightarrow$ *Chaos*

Therefore, to measure criticality in a particular network, one has to compute, either theoretically or numerically, the mapping $M(p)$ and then its slope S at the origin. If $S \approx 1$, the system is operating at, or close to, criticality.

CONTINUOUS MODELS OF THE SAP-ACTIVATED SIGNALING PATHWAY

As mentioned above, sea urchin sperm are able to locate and swim towards their conspecific eggs during broadcast spawning events. This remarkable chemotactic response is mediated by spatially and temporally organized spikes of $[Ca^{2+}]_i$, well documented through quantitative imaging of sperm swimming confined to a plane (Böhmer et al., 2005; Guerrero et al., 2010; Kaupp et al., 2003; Wood et al., 2005). The intensity of the Ca^{2+} influx predicts the turning curvature and the time elapsed between consecutive $[Ca^{2+}]_i$ spikes is organized such that the overall trajectory is chemotactic (Alvarez et al., 2012; Böhmer et al., 2005; Guerrero et al., 2010; Ramírez-Gómez et al., 2020; Wood et al., 2005); lack of coordination of spike intensity and intervals results in random, non-chemotactic relocation (Alvarez et al., 2012; Guerrero et al., 2013; Wood et al., 2007). This brings the spotlight to the control of the amplitude and timing of $[Ca^{2+}]_i$ spikes. These quantitative properties are beyond the scope of discrete models, like the one described in the previous section, and describing them requires quantitative models (see Box 1).

The first continuous model of SAP signaling was introduced by Aguilera et al. (2012) to demonstrate that a minimal network of speract-activated signaling pathway contained sufficient and necessary elements able to generate $[Ca^{2+}]_i$ oscillations comparable to the ones observed experimentally in *S. purpuratus* sperm stimulated with speract. The model was based on ordinary differential equations describing the ion fluxes and V_m in a Hodgkin-Huxley type of formalism. Parameter estimation was done by compiling values of about half of the 23 parameters from the literature and tuning the values of the remaining parameters such that numerical solutions of the model displaying periodic $[Ca^{2+}]_i$ oscillations with an amplitude and frequency with values comparable to those reported experimentally. The authors further compared the basal equilibrium $[Ca^{2+}]_i$ value in unstimulated sperm in the model to that reported experimentally which were the same order of magnitude. They further validated the model by showing it recapitulates a reduction in the frequency of the oscillations of $[Ca^{2+}]_i$ when sperm are activated with speract in the presence of the niflumic acid, assuming it inhibits the conductance of HCN, CaKC, CaCC, and HVA channels by percentages between 25% and 90%. Then, to assess the stochastic effects of the reduced number of channels, the authors implemented a hybrid stochastic-continuous simulation in which the number of open channels was drawn from the expected probabilities sampling from the corresponding binomial distribution. Finally, they individually blocked each ion flux "in silico" to find that the core oscillator is composed of Na^+, Ca^{2+}, and V_m. A limitation of this work was that, other than getting the simulated ion concentrations in the correct order of magnitude, the comparison of the numerical solutions and experimental time series was done by visual inspection with the authors noticing the "resemblance between the model results and those reported" (Aguilera et al., 2012). The other limitation was that CatSper was not in the set of Ca^{2+} channels of the model.

We overcame, at least partially, these limitations, in a study of the network elements involved in generating a characteristic pattern of decaying amplitude and increasing period of Ca^{2+} oscillations triggered by SAP in sea urchin sperm (Priego-Espinosa et al., 2020). We refined and extended the network previously proposed to feature CatSper, in addition to other voltage-gated Ca^{2+} channels, here denoted as Cavs. The model, presumably general, is assumed to describe the system in sperm from different sea urchin species. The extended network was partitioned into modules coupled by V_m: an upstream SAP-signal transduction module, and two Ca^{2+}-signaling modules, one structured around Cav-BK and another around CatSper-NHE channels. The carving of the network in this way was tactical. On the one hand, the modular structure allowed us to compare in an explicit, quantitative manner the numerical solutions of corresponding variables with different experimental measurements.Additionally, it facilitated the study of the modules alone or in combinations to ascertain the putative role of different channels. As in the previous work, many parameter values were taken from the literature or constrained to generate equilibrium values of $[Ca^{2+}]_i$, V_m and other variables, comparable to the ones reported. The predictions of the upstream SAP-signaling transduction module were fitted to cGMP and V_m time series reported in *A. punctulata* sperm populations stimulated with different doses of resact (Kaupp et al., 2003) before the effects of downstream elements came into play. The fitting required that upon SAP engagement the active guanylate-cyclase transits through high activity, to low activity and eventually inactive forms. Finally, the parameter space was systematically explored to find parameter sets in which the Ca^{2+}-signaling modules alone or combined could predict semiquantitatively the observed trends of decaying amplitude and increasing delay in consecutive Ca^{2+} spikes. We provided insights into the mechanisms generating these trends by treating the concentration of cGMP, the output variable of the upstream module, as a control parameter in bifurcation analyses of the dynamical behavior of the remaining network modules. Bifurcation analysis is the core of dynamical systems theory allowing to characterize how the asymptotic behavior of a model changes as a function of any chosen parameter. It identifies the existing dynamical attractors characterizing their nature (equilibrium state, limit cycle, strange attractor), their stability or instability in response to perturbations, and specific characteristics such as the amplitude or period of oscillations. The crux of the analysis is identifying the bifurcation points, i.e. critical values of the chosen parameter at which the predicted attractors come into existence, vanish or change their properties. Through bifurcation analysis, we

identified how the slow sustained decay in cGMP elicited by SAP leads to Hopf bifurcations that explained the transitions from stable equilibria to limit cycles, as well as the progressive decay in amplitude and increase in the period of the oscillations. The analysis showed that under appropriate parameter sets, both Cav-BK and CatSper-NHE modules could generate realistic $[Ca^{2+}]_i$ oscillations. The Cav-BK module generated acute spikes and a tonic plateau of $[Ca^{2+}]_i$ that resembled more the oscillations measured in speract-stimulated sperm than the blunt $[Ca^{2+}]_i$ peaks and deep nadirs predicted by the CatSper-NHE module. Intriguingly, the parameter exploration showed that the two modules tended to annihilate each other, as realistic Ca^{2+}-spike trains were obtained only with parameters where one module predominated over the other, the Cav-BK module tending to be more robust. To further inquire into the signaling network, we compared the two Ca^{2+}-signaling modules for the capacity to predict the effect of alkalinization on the $[Ca^{2+}]_i$ oscillations under the realistic parameter sets previously identified. The overlay of experimental and model-predicted $[Ca^{2+}]_i$ time series (Figure 2), led us to conclude that the model could only reproduce the observed abrogation of $[Ca^{2+}]_i$ spikes by alkalinization when the Ca^{2+} influx was mediated by a $pH_i/V_m/Ca^{2+}$-dependent mechanism, via CatSper, and not when it was mediated by a purely V_m/Ca^{2+}-dependent way, via Cav and BK channels.

The tribulations that both Aguilera et al. and Priego-Espinosa et al. went through to estimate parameter values illustrate well that parameterization is the major challenge when modeling signaling systems using differential equations (see Box 1). This is not a fundamental challenge, but a difficulty of principle. Hodgkin and Huxley (1952) solved the challenge almost a century ago by independently measuring in the giant squid axon system all the necessary parameters, from channel conductances through the parameters of all the gating functions.

An endeavor of a similar caliber to determine all the parameters of all the channels potentially involved in the response of sperm to SAP-stimulus has not yet been done, whether because experimental researchers have not found enough justification to do it or simply because it is not feasible in this biological system. Model builders may use strategies to circumvent this limitation, like estimating sets of parameters by model fitting, however, realistic models and their validation will require the measurement of the parameters by methods that are independent of model fitting.

What is rate limiting for further advances in quantitative modeling of sperm signaling is the experimental measurement of the parameters controlling channel dynamics and not the algorithms to integrate nonlinear models numerically or the computational solutions to assess model robustness in large parameter spaces. The latter can be found off-the-shelf these days. To be clear, experimentation and measurements are the main challenges for future progress in modeling. On theoretical grounds, the main challenge is likely the integration of SAP-signaling models with the biophysics of sperm swimming. Only when such integrative models are developed and studied will one understand quantitatively how the signaling transduction mechanisms steer the swimming of cells to produce chemotactic or non-chemotactic behaviors. Multilevel modeling and analysis of complex data sets including measurements at molecular, cellular and organismal levels are hot topics for today's systems biology.

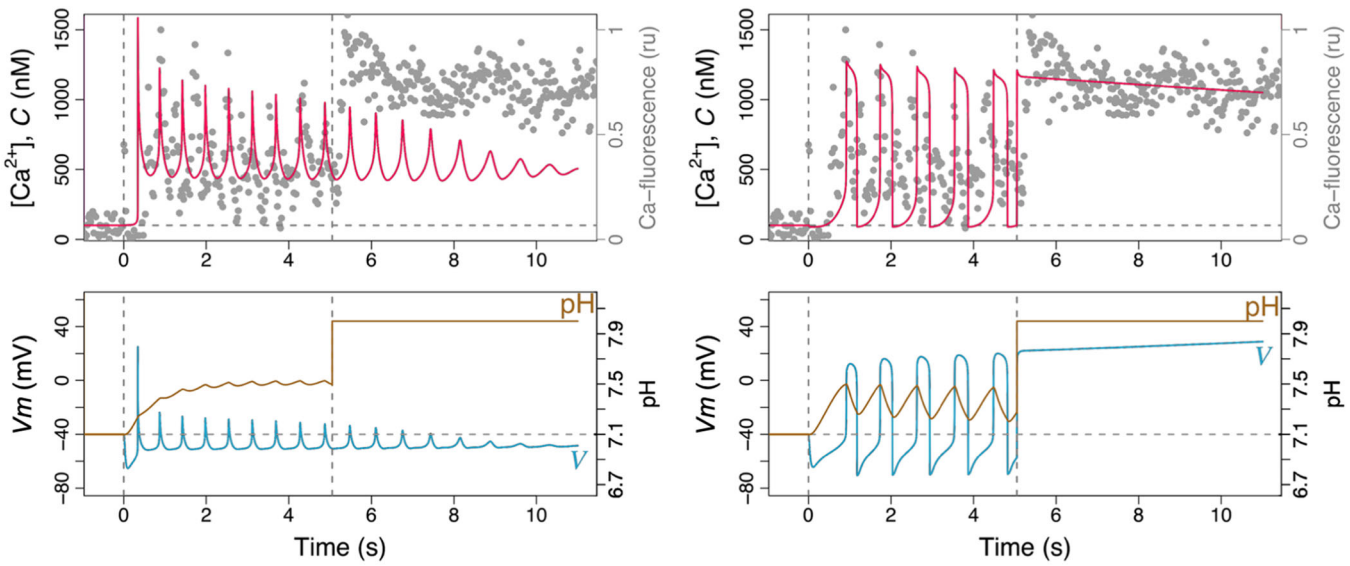


FIGURE 2 Continuous model of SAP-triggered Ca^{2+} oscillations in sea urchin sperm flagellum. Comparison of the numerical solutions of the model featuring the Cav-BK (left) and CatSper+NHE (right) modules before and after forced alkalinization. The flagellar $[Ca^{2+}]_i$ (red), membrane potential (V_m) (blue) and pH_i (brown) time course in the model are plotted as a function of time. A rescaled experimental time series of experimentally measured Ca-fluorescence (gray dots) is overlaid with the predicted $[Ca^{2+}]_i$ time series (adapted from fig. 9, Priego-Espinosa et al., 2020).

MODELING MOUSE SPERM CAPACITATION

Unlike external fertilizers, such as sea urchins, sperm in mammals must reside inside the female genital tract for some time (minutes to hours) to complete a unique maturation process known as capacitation. This process primes the physiological state of sperm to allow them to find, fuse and fertilize the egg at the right time and location. Capacitation, independently discovered by Austin and M.C. Chang in the 1950s (Austin, 1951; M. C. Chang, 1951), involves orchestrated electrochemical changes and membrane remodeling that prepare the sperm for the AR, a Ca^{2+} and pH-dependent regulated exocytotic process that mediates egg-sperm fusion. Additionally, capacitation leads to hyperactivation, an asymmetrical whip-like pattern of flagellar beating that influences swimming behavior depending on media viscosity (H. Chang & Suarez, 2010). This change facilitates the navigation inside the complex and viscoelastic female reproductive tract. In vitro fertilization requires the incubation of sperm in a chemically defined medium that mimics the environment of the genital tract. This alkaline medium, known as capacitation medium, has precise concentrations of $CaCl_2$, NaCl, $NaHCO_3$, KCl, cholesterol acceptors, and metabolites (reviewed in Toyoda & Yokoyama, 2016). However, only a fraction of the cells undergoes capacitation under capacitating conditions, and that is the fraction able to achieve AR. It is still not fully understood why only a fraction of sperm fully reach capacitation and whether this heterogeneity entails reproductive benefits.

Sperm, which are highly specialized, are basically unable to express genes. They have signaling networks that leverage electrochemical gradients and bring about regulated changes in V_m and $[Ca^{2+}]_i$, pH_i, and other second messengers. The concerted action of ion transporters, receptors, and enzymes relay those biochemical and biophysical signals (Darszon et al., 2011). Unsurprisingly, this sperm signaling motif is to some degree maintained in all mammals. In mice, a preferred model, sperm capacitation involves V_m hyperpolarization (Escoffier et al., 2015), rises in $[Ca^{2+}]_i$ (Wennemuth et al., 2003), pH_i (Darszon et al., 2011), $[Cl^-]_i$ (Hernández-González et al., 2007), and a decrease in $[Na^+]_i$ (Escoffier et al., 2012). Then, these signals trigger PKA-dependent phosphorylation within the first few minutes of capacitation, followed by tyrosine phosphorylation cascades over longer time scales (Visconti, Bailey, et al., 1995; Visconti, Moore, et al., 1995) and increased actin polymerization (Breitbart et al., 2005; Brener et al., 2003).

The intricacies of how physiological processes are interconnected in capacitation make it a suitable subject for mathematical and computational modeling with a systems biology outlook. The available modeling research on murine capacitation signaling can be categorized by whether the focus is on the architecture (topology) or the dynamics of a portion of the signaling network.

Network architecture studies in murine capacitation signaling

The initial attempts to create comprehensive networks for capacitation were conducted by Bernabò et al. They reviewed the literature relevant to capacitation and manually constructed networks. The set of network nodes is defined by hundreds of molecule types and physiological processes, while the set of pairwise interactions consists of functional and physical associations between them. Some networks were built for particular species, like human (Bernabò et al., 2010, 2019), and boar (Bernabò et al., 2017; Bernabò, Berardinelli, et al., 2011), while others were more focused on general questions and merged information from several mammalian species to create a "chimeric" network (Bernabò, Greco, et al., 2015). If we adopt a looser definition of capacitation and consider epididymal maturation to be a part of it, the first integrative network model for a part of mouse sperm capacitation was proposed by Bernabò et al. (2016), which addresses membrane remodeling and lipid metabolism while sperm travel through the epididymis.

The usefulness of this kind of approach is supported by two premises. On the one hand, inquiries based on the quantification of certain node connectivity properties can offer insights about the importance of a signaling element, for example, is it a highly connected node (hub)?, how interconnected are its immediate neighbors (high clustering coefficient)?, could it serve as a bridge in the information flow between two clusters (high betweenness centrality)?, what would be the possible consequences of removing such a relevant node for the signaling throughout the whole network?

On the other hand, networks can be characterized by the global patterns of their node connectivity (node degree) and their clustering coefficients; within the framework of network statistical mechanics, distributions of these measurements can help classify signaling networks as for example, random, scale-free or small-world, each with particular implications. For instance, scale-free networks (Albert & Barabási, 2002), like the one constructed by Bernabò et al. (2016), are known for their robustness against random node failure, controllability concentrated in a few hubs, and reduced loss of information. In the network analysis performed by Bernabò et al. (2016), cholesterol is identified as a network hub and stands out in terms of other connectivity parameters, along with proteins involved in lipid trafficking to a lesser extent.

Network dynamics of murine capacitation

Discrete dynamics

In Aguado-García et al., we explored the hypothesis that the heterogeneity in the capacitation response could stem from the variability in the ion transporter counts per sperm cell (2021). A multitemporal discrete dynamics network model was devised to simulate the early capacitation of murine sperm, which involves electrochemical events through PKA activation (Figure 3a). The model ignores the tyrosine phosphorylation cascade that takes place at late stages and only considers the signaling events in the flagellum without including spatial aspects.

The dynamical rules of a subset of the nodes followed a classical discrete synchronous formalism as in Kauffman networks (see Box 1),

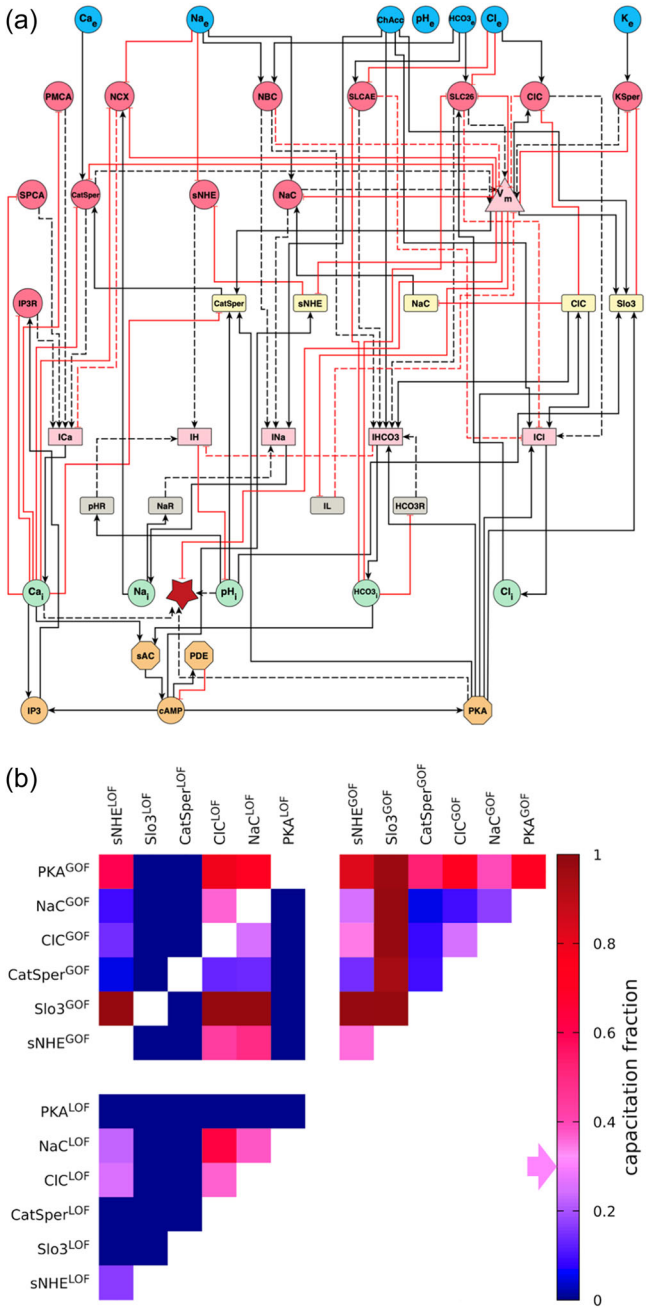


FIGURE 3 Discrete regulatory network model of early capacitation in mouse sperm. (a) Wiring diagram with nodes and edges styled according to functional categories. The node types are: extracellular ion concentrations (blue circles), intracellular ion concentrations (green circles), electromotive force of ion transporters (pink circles), ion transporter gates (yellow boxes), net ion fluxes (light pink boxes), membrane potential (V_m) (light pink triangle), enzymes involved in cAMP and phosphorylation signaling (orange octagons), intracellular concentrations of non-ionic second messengers (orange circles), and auxiliary variables involved in restoring resting V_m and ion concentrations (gray boxes). A special auxiliary node that measures the collective dynamics of variables representing capacitation signatures is depicted as a red star. The edges are: activating (black edge, ending with an arrowhead) or inhibiting (red blunt-ended lines), and participating in a logical (solid) or threshold (dashed line) dynamical rule. (b) Fraction of capacitated sperm after single or double in silico mutations. The fraction of capacitated sperm in silico is obtained by repeating simulations with different initial conditions as described in the original publication. The arrow indicates the capacitation frequencies of 30 ± 5% observed in wild-type sperm stimulated in capacitation conditions in vitro and reproduced by the model. The values of the gating variables of 4 ion transporters and PKA were fixed to constant values of 0 or 1 to model loss-of-function (LOF) or gain-of-function (GOF) mutations, respectively. Reproduced and modified from Aguado-García et al. (2021).

similar to Espinal et al. (2011). However, we had to extend this model with the help of other tools to account for more complex features that characterize capacitation, like the response heterogeneity and different time scales operating along the signaling transduction. For this endeavor, their ad hoc network model integrated different discrete formalisms and included stochastic components (see hybrid models in Box 1). Hence, it is worth highlighting some of the most interesting challenges posed to us by the varying levels of complexity of the early capacitation response and how such issues were addressed (Aguado-García et al., 2021).

(a). Signaling input/output complexity. Unlike the SASP, which has a single input (speract) and a single output variable ($[Ca^{2+}]_i$) whose periodicity is of interest, the capacitation response depends on several input variables defined by the capacitation medium. As this model is circumscribed to the early stages of capacitation, the output is reported by an auxiliary variable (see star node in Figure 3a) that captures how frequently the state of 4 variables coincides with the signature of capacitation (elevated $[Ca^{2+}]_i$ and pH_i, hyperpolarized V_m, activated PKA), and also assesses if such desired states converge at a rate that exceeds a predetermined threshold within a defined timeframe. It is worth noting that if the model had included the entire capacitation response and AR, the output would have been a binary variable tracking the completion of the AR.

(b). Discretization of electrochemical quantities. The number of regulators and states when constructing truth tables for the nodes involved in ion fluxes posed a combinatorial explosion that is difficult to manage. This issue was facilitated by discretizing analytical expressions (algebraic and differential equations, which take values in the real numbers set) for electrochemical potentials, ion channel gating, and ion transporter activities. As for how ion concentrations update their state depending on the ion flux, auxiliary nodes concentrate the sum of fluxes coming from different ion transporters and discretize the resulting quantity into either of 3 values depending on its sign (−1, 0 or 1), which are associated with the direction of the net flux. The latter resource is an example of threshold Boolean logic formalism (Bornholdt, 2008; Zañudo et al., 2011), which is inspired by the neuronal network model developed by McCulloch and Pitts (1943) (see Box 1). The V_m node was approached in a similar

manner but with threshold values that differ from those used in the sign function. These thresholds were parameterized to establish the resting level when capacitating stimulus exists (Aguado-García et al., 2021).

(c). Heterogeneity in ion transporter counts. In the context of ion fluxes and V_m nodes, weight parameters used in the neuronal network-like approach represent an aggregate quantity that includes both the unitary conductance and the ion transporter count. These weight parameters regulate the influence of each transporter type over their regulated nodes. To account for the differences in the number of ion transporter counts due to variability in gene expression during spermatogenesis, and to acknowledge that the resulting protein stoichiometries remain stable during sperm lifetime, each weight was multiplied by a random variable that samples a number from a normal distribution, once in every individual network realization. This guarantees that each replicate (individual sperm) has its own unique set of ion transporter counts and adds a probabilistic aspect consequently.

(d). Asynchrony. In view of the different activity and response rates for the molecule types and cellular processes considered in sperm capacitation, the synchrony assumption implicit in boolean networks becomes far-fetched. The model uses interaction-wise discrete influence rates similar to kinetic constants in differential equations. Specifically, for each interaction, a Bernoulli trial (or "coin-tossing") is performed wherein the above-mentioned rate is the probability of a node participating in the updating function of its regulated node. This adds a stochastic element to an otherwise deterministic asynchronous scheme. For most ion transporter nodes, when the participation turns out null after drawing the corresponding random number, their state is set to the least active state. For ion fluxes coming from a different cell compartment (i.e., the redundant nuclear envelope [RNE] in the sperm neck) and other elements like PKA phosphorylation and cholesterol removal, the nonparticipating state is set to that of the previous time step. This introduces a delayed dynamics effect and accounts for diffusion processes. Overall, this novel modeling feature allows for the introduction of various time scales in a simplified manner.

To better infer the molecular causal chains in the capacitation response, an ideal experimental setup would imply tracking multiple physiological variables simultaneously in an individual sperm, from the start of the capacitating conditions until its ability to undergo AR is tested. However, the current cell physiology techniques are still limited in that respect. Despite this, we proposed a criterion to classify single sperm cells based on the joint dynamics of multiple variables in early capacitation simulations. Furthermore, our research explored the parameter space that defines such capacitation criterion and found regimes that reproduce capacitation levels comparable to the empirical observations as well as distributions of physiological variables compatible with flow cytometry measurements (Escoffier et al., 2015; Luque et al., 2018). One of the crucial parameters in those regimes was related to the degree of ion transporter count variability, which led to the main finding of the study: the distribution of ion transporter counts could regulate capacitation and underlie the typical heterogeneity in such response.

With a reliable parameter set to reproduce capacitation heterogeneity in hand, we simulated various network variants that introduced in silico single or double mutations in selected nodes. We studied two types of mutations: loss-of-function and gain-of-function (Figure 3b). With that analysis, our aim was to either reproduce results previously reported in the literature or generate hypotheses to be tested by experiment.

Continuous dynamics

With the help of a reaction-diffusion model, which is a type of differential equations model wherein spatial considerations can be modeled more explicitly by means of diffusion terms, Olson et al. (2010) addressed the empirical findings of a tail-to-head Ca^{2+} propagation followed by a sustained $[Ca^{2+}]_i$ elevation observed in the mouse sperm head, during a hyperactivation Ca^{2+} response induced by a cAMP analog (Xia et al., 2007). Those experiments showed that the sustained $[Ca^{2+}]_i$ elevation was triggered by the Ca^{2+} influx through CatSper channel complexes, located along the principal piece of the flagellum.

Olson et al. (2010) tested two hypotheses to explain such observations. In one simple model, they only considered Ca^{2+} fluxes through the plasma membrane: CatSper, Ca^{2+}-ATPase pump (PMCA), and a passive leak influx term. The second scenario extended the simple variant to include Ca^{2+} fluxes from another cell compartment, namely, the RNE. Even though both scenarios reproduced the tail-to-head propagation, only the second one managed to generate a sustained $[Ca^{2+}]_i$ in the head. This was proposed to be mediated by a Ca^{2+}-dependent upregulation of inositol 1,4,5-trisphosphate (IP_3) synthesis and the ensuing Ca^{2+} release from a reservoir in the neck through an IP_3^-gated Ca^{2+} channel.

Just as Aguilera et al. (2012) focused on finding a minimal variable set underlying the stereotypical Ca^{2+} oscillatory behavior in the SASP, de Prelle et al. developed a minimal model to study the likely bistable switch coming from the positive feedback loop between sNHE and SLO3 (2022). This hypothetical feedback loop, suggested by the experimental work of Chávez et al. (2014), was thought to be the natural consequence of two complementary findings: SLO3 is a pH-dependent K^+ channel that hyperpolarizes the membrane, while sNHE is a hyperpolarization-dependent transporter that raises the pH_i by extruding protons.

The model devised by Prelle et al. consisted of an ODE system for V_m and pH_i dynamics, which had external bicarbonate ($[HCO_3^-]_e$) as a stimulus (2022). Their study was based on a simpler concept of capacitation, membrane hyperpolarization and cytosol alkalization of a single sperm. With this concept, they performed a bifurcation analysis that had $[HCO_3^-]_e$ as a control parameter, wherein the two resulting steady stable states were capacitated and non-capacitated,

respectively. Bistable switches like this are well known for having hysteresis, a property of the system in which for a certain range of stimulus (in this case, $[HCO_3^-]_e$), two stable states coexist and are separated by an unstable one. This property presents three important consequences: depending on whether the sperm came from a low or high $[HCO_3^-]_e$, it would end up in one of these stable states; the sperm would be locked in either state as long as the external stimulus fluctuations are small; capacitation reversal would be possible, at least in terms of their simplified criterion defined by just two variables.

Interestingly, when they compare the relevance of sNHE and SLO3 by testing how the capacitation response holds at several $[HCO_3^-]_e$, stimuli when either of those transporters is inhibited, they come to the conclusion that the blockage of SLO3 is more effective and robust at hindering capacitation than inhibiting sNHE3. This is compatible with the discrete dynamics simulations by Aguado-García et al. (2021), in which they predict that the capacitation inhibition due to SLO3 loss-of-function mutation could not be rescued by a gain-of-function mutation of sNHE, whereas the opposite scenario is not true, given that SLO3 overactivation takes sperm population to nearly complete capacitation levels in spite of sNHE blockage (see Figure 3b). Combining these two studies suggests that SLO3 may play a central role in controlling fertility in both directions.

Limitations for the existing models and future directions

Different models take distinct approaches to determine which elements are relevant to a research problem. This typically involves excluding certain parts of the system to make it more tractable. For instance, Aguado-García et al. (2021) concentrated on electrophysiological considerations and PKA-dependent phosphorylation in the flagellum during early capacitation. Their model did not account for the characteristic tyrosine-dependent phosphorylation patterns that appear after long periods of incubation, lipid and actin dynamics during membrane remodeling of the head, or metabolic changes. Similarly, Olson et al.´s model (2010) explains the spatiotemporal dynamics of the cAMP-triggered Ca^{2+} increase, but only addressed Ca^{2+} transporters, a couple of second messengers (IP3 and cAMP), and Ca^{2+} itself, disregarding the effect of V_m, pH_i, and other types of ion transport. On the other hand, the minimal model of de Prelle et al. (2022), meant to explore a bistable switch given by the dynamics of two variables (sNHE, SLO3), only modeled the dynamics of pH_i and V_m, without the inclusion of other ion transporters or signaling elements; nevertheless, they acknowledged the possibility of another switch that results from the positive feedback between CatSper and sAC.

When it comes to extending a model to include more processes and elements, the mere addition of nodes would not represent a significant challenge in a network-topology-centered study, as opposed to calibrating the rules that govern the time evolution of each new variable in a dynamical model. However, it is important to be cautious when examining how the network structure relates to information transmission. Recent studies have shown that effective connectivity is superior to simple connectivity when it comes to understanding this relationship. Effective connectivity is a concept that considers the importance of a regulator node in determining the final output of the dynamical function of its regulated node (Gates et al., 2021; Manicka et al., 2022).

Another unexplored part in capacitation dynamical models is the mitochondria, which in mouse sperm constitute large complex subcellular structures with both Ca^{2+} dynamics and energy production capabilities. Although past models (Aguado-García et al., 2021; Olson et al., 2010) neglected this organelle for being unable to fully bring $[Ca^{2+}]_i$ back to resting levels (Wennemuth et al., 2003), recent findings suggest its significance should be reconsidered (Sánchez-Guevara et al., 2023).

At the present time the need for complexity to achieve capacitation is being debated, considering recent results from the human sperm model (Grahn et al., 2023). It will be interesting to see, as more local and global properties come to focus in the research of the coming decades, what the essence of capacitation is and how general it is species-wise. Certainly, an integrated view of this process is required which will emerge as new experimental and modeling strategies develop. For example, the insights of Aguado-García et al. (2021) will require measuring ion channel stoichiometries in single cells.

MODELING THE ACROSOME REACTION IN MAMMALIAN SPERM

The journey of sperm toward fertilizing an egg is marked by numerous physiological transformations, one of which is the acrosome reaction (AR). This crucial biological event occurs when the acrosome, an acidic vesicle located at the apical part of the sperm's head, undergoes a series of changes that allow the sperm to penetrate and fertilize the egg (Bianchi & Wright, 2016). This exocytotic process results in the release of hydrolytic enzymes into the extracellular medium, along with the reshaping and reorganization of the plasma membrane (Cohen et al., 2016; Dan, 1952, 1954). These changes allow sperm to advance in the female genital tract and reach the zona pellucida (ZP) — a glycoprotein layer enveloping the oocyte. The AR transforms the sperm plasma membrane to facilitate attachment and passage through the ZP, as well as docking and fusing with the egg's plasma membrane — steps that are critical for successful fertilization (Darszon et al., 2011; Florman & Ducibella, 2006; Jin et al., 2011; Okabe, 2018).

Beyond the need to further understand the mechanisms of the AR, this knowledge will impact our ability to control male fertility, diagnose its problems and find solutions (Kizilay & Altay, 2017; D. Y. Liu & Baker, 1994; Oehninger, 2000; Pampiglione et al., 1994; Zeginiadou et al., 2000). A deeper understanding of the AR will also contribute to better comprehending membrane fusion, a key phenomenon for cell function (Romero et al., 2024). Diverse animal

models, going from flies and sea urchins, up to mammals, allow contrasting similarities and differences in reproductive strategies (Darszon et al., 2020; Hirohashi & Yanagimachi, 2018).

In mammals, the AR requires capacitation, as explained earlier. The primary physiological triggers of AR in vivo, depending on the species, are believed to be the ZP glycoproteins (notably ZP3 in humans) (Florman & Ducibella, 2006; O'Toole et al., 2000) that surround the egg, and progesterone (Harper et al., 2006); they can initiate AR in vitro. However, recent studies in mice have indicated that the AR is triggered before ZP contact, therefore it is necessary to reevaluate other physiological agonists (Jin et al., 2011). Progesterone is secreted by cumulus cells near the oocyte, thus it is now considered a viable candidate (Bronson, 1999; Harper et al., 2006), although how it triggers AR remains unclear.

The AR requires a complex set of orchestrated molecular sperm events, including the elevation of $[Ca^{2+}]_i$ (Darszon et al., 2011; Sosa et al., 2016) and pH_i (Balbach et al., 2020; Nakanishi et al., 2001), phosphorylation changes in several proteins (Ickowicz et al., 2012; Zhang et al., 2022), the activation of the membrane fusion pathways (Mayorga et al., 2007; Sosa et al., 2014), plasma membrane hyperpolarization (Darszon et al., 2011; De La Vega-Beltran et al., 2012) and cytoskeleton restructuring (Breitbart et al., 2005; Romarowski et al., 2018). Molecular players such as enzymes, ion channels, and structural proteins coordinate this process whose culmination is the fusion of the external acrosome membrane with the posterior head sperm plasma membrane and the release of acrosomal contents.

The role of $[Ca^{2+}]_i$ in the AR is well-documented. It undergoes a biphasic response: a rapid initial $[Ca^{2+}]_i$ spike within the sperm head, followed by a stabilization phase at a higher plateau (Kirkman-Brown et al., 2000; Patrat et al., 2000). Investigating the AR in real time is challenging as it displays complex fast and slower asynchronous phases of multiple variables among the sperm population. As previously commented, it is known that only a small fraction of the sperm population achieves AR, a heterogeneity problem that further compounds its overall understanding. In addition, a complete picture of the various physiological triggers and molecular pathways involved in this unique exocytotic process is lacking. Differences in experimental conditions across various studies have also led to inconsistent findings that preclude arriving at conclusive insights.

In this context, mathematical modeling emerges as a potent tool to study the AR, offering a means to synthesize experimental data into a cohesive framework. Such models can capture the dynamics of the AR, accommodating the various biochemical and cellular components involved. By establishing parameters based on experimental data, mathematical models can simulate the conditions under which the AR occurs and predict the system's behavior in response to different stimuli. These models can also test hypotheses about the roles of specific molecules and pathways in the AR, which may be challenging to evaluate experimentally.

Simons and Fauci (2018) constructed a mathematical model of the $[Ca^{2+}]_i$ regulatory dynamics during the AR, revealing some novel aspects relevant to this process. Based on established mathematical frameworks for Ca^{2+} dynamics in various cell types, this model was tailored to replicate the unique biphasic $[Ca^{2+}]_i$ response seen in the AR. By focusing on temporal dynamics, the model aligns with experimental fluorescence microscopy records of $[Ca^{2+}]_i$ changes in the sperm head. It also includes additional elements of this process in human sperm. This dual approach provides a deeper understanding revealing insights about the complex nature of the AR.

The model hints at the potential importance of the dynamics of protein kinases PKC and PKA in modulating the sustained $[Ca^{2+}]_i$ elevation that plays a pivotal part in triggering downstream events essential for membrane fusion and acrosomal release. Simons and Fauci (2018) propose that feedback mechanisms, such as the inhibition of phospholipase C (PLC) by PKC, may dampen the oscillatory $[Ca^{2+}]_i$ behavior, ensuring a stable and appropriate cellular response. They propose that sperm have evolved to use multiple pathways to ensure the fidelity of the AR. The complexity and sensitivity of this system are highlighted by the fact that even slight variations in the biochemical pathways and feedback mechanisms can lead to divergent outcomes.

Although the current model is simplified and omits several factors like spatial effects, Ca^{2+} channel voltage-dependence, and pH_i changes, it still captures the qualitative behaviors of Ca^{2+} responses observed experimentally.

More recently, in A. Aldana et al. (2021), we proposed a novel discrete mathematical model, capturing the intricate physiological interplay regulating the AR in human sperm. This comprehensive model, though still partial, incorporates various components that are critical in regulating the AR, accounting for the dynamics of V_m regulation, the concentrations of Ca^{2+} both in the cytosol and the acrosome, the regulation of pH_i levels, the control mechanisms of membrane fusion, and the effects of external stimuli on these processes. Interestingly, the model shows the role of intra-acrosomal components as pH and Ca^{2+}, in the development of the AR.

This study employs a generalized version of the gene regulatory network (GRN) framework. The main signaling pathways involved in the AR are formally translated into a regulatory network. Here, each node represents one of the primary components of the AR signaling pathway, and the links depict regulatory interactions among these components (Figure 4a). To describe and analyze the dynamic properties of this model, the state of the entire network is characterized by a set of discrete variables, with each variable representing the state of a single node (see Box 1).

The analysis is focused on the dynamical properties of the model's attractors. We identified multiple attractors that can be classified in terms of their biological interpretation (see Box 2). This classification implies these attractors can represent the dynamical behavior of sperm that react spontaneously, through progesterone, or not at all (Figure 4b).

In delving deeper, the study examines the network states that lead to these varied attractors, calculating the activity patterns of the biomolecules involved in the AR. A significant discovery is the direct correlation identified between the proportion of sperm undergoing the AR and the structure of the population's physiological

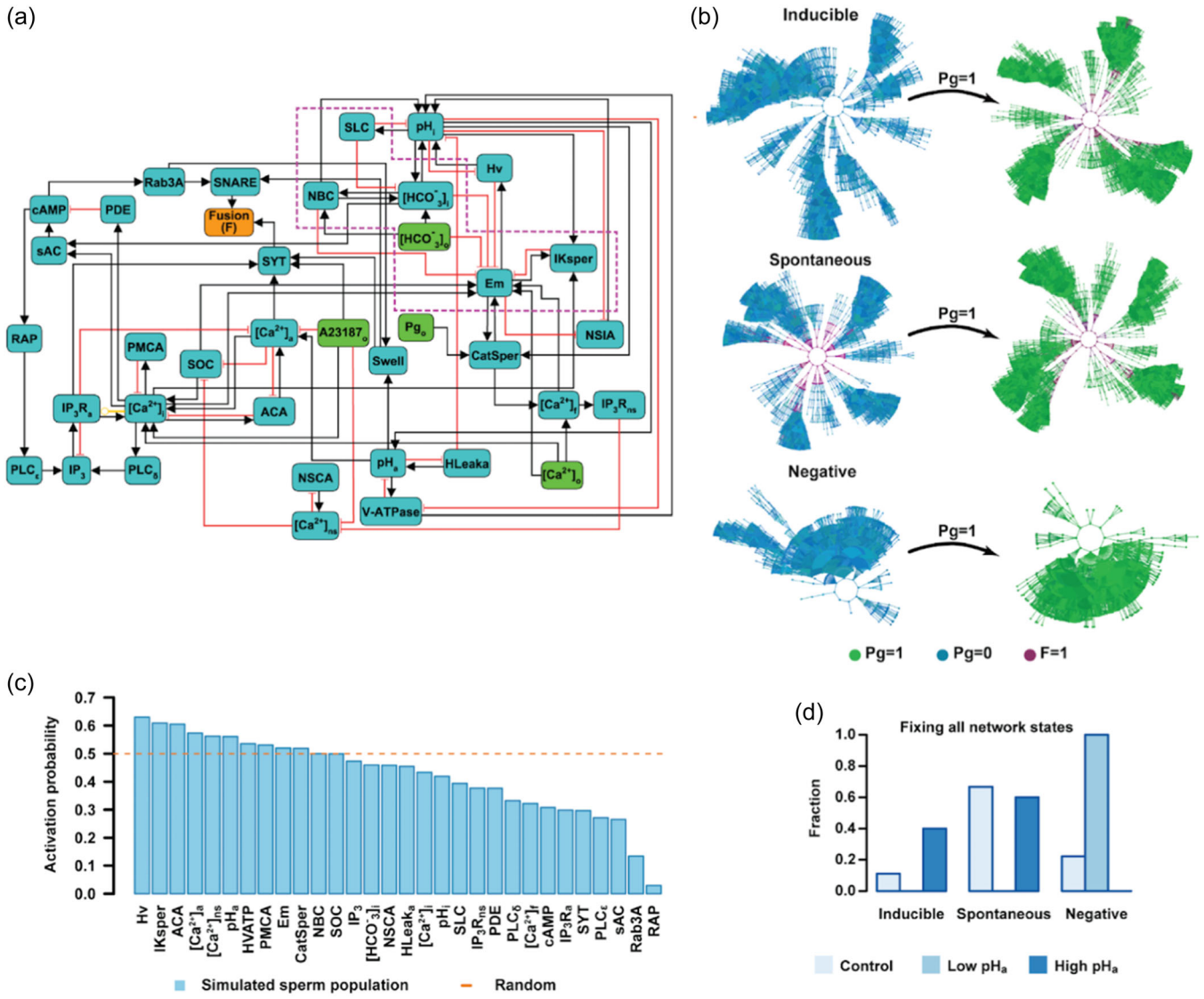


FIGURE 4 Discrete regulatory network model of the acrosome reaction in mammalian sperm. (a) Wiring diagram of the regulatory network for the acrosome reaction (AR) in human sperm indicating the nodes and interaction edges. (b) Network attractors, represented by state transition graphs, were classified based on their response to changes in the variable representing the presence of progesterone (Pg) in the extracellular medium. This classification aligns with experimental observations of sperm subpopulations that react spontaneously, react to Pg induction, or do not react at all. (c) Physiological heterogeneity is illustrated by the probability of each network node being activated in simulations of sperm populations (blue bars). These simulations reproduce experimental proportions of spontaneous and Pg-induced AR. Deviations in activation probabilities can lead the model to diverge from experimental proportions. (d) Perturbation analysis demonstrates that overactivation of the intra-acrosomal pH node (indicating a pH_a elevation) results in an increased number of attractors representing spontaneous and Pg-induced AR. Conversely, underactivation of this node (indicating acrosomal acidification) leads to a reduced number of attractors capable of reaching the AR state. All panels are taken and adapted from A. Aldana et al. (2021).

heterogeneity (Figure 4c). This correlation is crucial to understanding how this heterogeneity influences both spontaneous and progesterone-induced AR, shedding light on the underlying mechanisms of sperm reactivity.

By addressing the heterogeneity observed in sperm populations, the model attempts to predict the AR's probability under varying conditions. This model provides insight into how the collective behavior of numerous interacting components results in a biological outcome. The absence of fixed-point attractors (steady states) in the system suggests that the AR is characterized by stable oscillations, implying that the timing and initiation of the reaction are intrinsically regulated by the sperm's internal network rather than solely by external stimuli.

We demonstrated that the model can qualitatively replicate a variety of experimental observations and offers a means to analyze the regulatory functions of acrosomal pH (pH_a) and cell heterogeneity during the AR. By applying single-node perturbations that affect the dynamical properties of the network attractors, the authors confirm

that an increase in pH_a can initiate AR in a sperm population and highlight its essential role in acrosomal exocytosis stimulated by progesterone, a well-known natural inducer of the AR (Figure 4d).

Collectively, the findings accentuate that physiological heterogeneity is intimately connected to the proportion of sperm undergoing AR, and establish an increase in pH_a as a pivotal event in the unfolding of the AR in humans.

The two models presented have significant constraints and simplifications, such as omitting certain molecules involved in regulating the AR, employing discrete variables, assuming synchrony, and failing to consider the stochastic nature of biological phenomena. Nonetheless, the use of mathematical modeling offers the potential to deepen our comprehension of the AR by enabling the investigation of theoretical realms that may elude direct empirical study. Consequently, this approach can contribute to the generation of novel hypotheses and the strategic planning of experimental work in the field of reproductive biology.

A CRITICAL APPRAISAL, OPEN QUESTIONS AND PROSPECTS

Electrophysiological signaling modeling has been traditionally dominated by continuous variable models, using ODEs or analog electric models following the Hodgkin and Huxley (1952), FitzHugh (1961), and Nagumo et al. (1962) exemplars. The modeling of sperm signaling is at odds with this well-paved tradition, as this review shows. The scarce number of ODE models in the literature, all focused on quantitative details of $[Ca^{2+}]_i$ oscillations in sea urchin sperm (Aguilera et al., 2012; Priego-Espinosa et al., 2020), is dwarfed by the reports analyzing sperm signaling with discrete logical network models (Aguado-García et al., 2021; A. Aldana et al., 2021; Espinal et al., 2011; Espinal-Enríquez et al., 2014, 2017; Guerrero et al., 2013).

Large number of parameters or very large truth tables: "No free lunch"

The greatest difficulty of implementing and analyzing continuous ODE models is the absolute necessity of specifying the values for a large number of parameters. ODE modelers found heuristic strategies to circumvent the dearth of parameter measurements, from amalgamating the information available on different species and experimental settings to resorting to efficient algorithms to explore large parameter spaces. The alternative and quite popular route is to put aside continuous models altogether and adopt discrete logical dynamics formalisms that do not involve parameters with units.

However, discretizing sperm electrophysiological signaling has proven challenging and the difficulties are not made explicit by the wire diagrams of state-transition graphs in publications. The curse of high-dimensional parameter spaces in the continuous framework translates to long convoluted logical functions or very large truth tables in the discrete logical framework. To make this argument clear, consider the representations of V_m. In Priego-Espinosa et al. (2020), V_m dynamics is described by the right-hand side of an ODE featuring 5 variables (measuring the open fractions of 5 ion channels) and 10 parameters. In A. Aldana et al. (2021), the V_m node dynamics is determined by a truth table involving 7 nodes (describing the state of 7 channels) and 384 inputs representing all possible permutations of the states of those nodes. As the popular adage says also here "there is no free lunch".

The challenge of modeling transient dynamics with a formalism without explicit time

Discrete dynamic models were first implemented to analyze transcriptional gene regulatory networks (GRN) by Kauffman (1969) and Thomas (1973) with the aim of predicting potential attractors that were interpreted as cell differentiation states. Most of the GRN analyses have been centered on identifying and characterizing the asymptotic fixed points and cyclic attractors. Nonetheless, extrapolating such analyses to understand transient responses is a nontrivial matter, especially if one considers that essential cell capabilities, like memory and fast adaptation to nonstationary external cues, could take place far from attractors (Koch et al., 2024). Experimental works on sperm physiology have pointed in that direction (Cohen-Dayag et al., 1995; Kashikar et al., 2012; Jikeli et al., 2015).

On the other hand, time is not made explicit in GRN as logical parameters have no time units and only the sequence of events leading to state transitions is relevant. Therefore, using this modeling formalism to describe the transient kinetics of the ion-signaling responses in sperm, which are highly differentiated transcriptionally inactive cells, is simultaneously novel and challenging.

The first obstacle is how to compare the results of a discrete model with the experimental time series of ion concentrations in sperm. Hitherto, these comparisons have been made in a qualitative ad hoc manner, relating the observed sequence of peaks and nadirs in ion concentrations to the sequence of discrete values of variables in the models (Espinal-Enríquez et al., 2014, 2017), precluding an objective assessment of the quality of the predictions. Efforts have been made to map modeling results to transcriptional time series in other cell types notably by inferring the underlying GRN from transcriptional time series (Bulashevska & Eils, 2005; Li et al., 2023; Marbach et al., 2012; Martin et al., 2007; Pušnik et al., 2022). The latter involves an estimation of the concentration-discretization thresholds for each node simultaneously with the times at which transitions happen. In sperm, however, this explicit and objective comparison has not yet been performed, remaining an open field for exploration.

The second challenge relates to time scales. Most studies of sperm signaling in sea urchin (Espinal-Enríquez et al., 2014, 2017) or mammalian (A. Aldana et al., 2021) sperm using discrete dynamics models, were systematically based on synchronous updating

dynamics. The synchronous updating dynamics assumes that the concurrent changes in the activity level of any molecular component happen simultaneously with the same time scale. Though this assumption makes the system deterministic and easy to simulate, it is a strong simplification of the temporal richness of ion flux dynamics. Several more realistic solutions have been proposed, ranging from asynchronous updating dynamics (Thomas, 1991), association of specific rates, delays or priorities to the edges in the state transition graph (Fauré et al., 2006; Siebert & Bockmayr, 2006) and simulation with the Gillespie algorithm (e.g., Stoll et al., 2017) or combination of the later with explicit delays (Aguado-García et al., 2021). However, a more systematic exploration of the robustness of the results to the specific choices of temporal dynamics of ion fluxes remains an open problem.

The challenge of comparing qualitative variables with quantitative ion concentrations and membrane potential measurements

Comparing continuous variables in ODE models to quantitative measurements is straightforward as it can be done directly if the units are the same or using some scaling factor as in Figure 2. When dealing with discrete variables in qualitative models, one usually discretizes the experimental measurements as mentioned before. In Espinal-Enríquez et al., (2014, 2017) a different approach was followed, transforming the model's discrete values into quasicontinuous values for comparison with observations. To this end, we averaged several thousands of simulations with random initial conditions. The aggregate time series results from the probability distribution of the underlying time series and the phases of each series that are conditioned by the probability distribution of the initial states. This is arguably meaningful when comparing the model with $[Ca^{2+}]_i$ quantification in populations but questionable when comparing with single-cell measurements. Furthermore, the assumption of equiprobable initial states is an approximation, and therefore the predicted outcomes depend on simple assumptions about the states of the individual components.

Despite the aforementioned challenge, it is worth noticing that the results on the Ca^{2+} dynamics based on a discrete regulatory network, reproduced a previously reported oscillatory behavior, and at the same time, it predicted the Ca^{2+} behavior after removing certain channels. This highlights the importance of an experimentally based regulatory function construction, as well as the robustness of the constructed model.

Quantifying the basins of attraction of cellular states

Drawing random initial values based on some probability distribution across a network is a common way of quantifying basins of attraction in GRN using synchronous dynamics. The AR model we reviewed above (A. Aldana et al., 2021) allowed us to address the probability distributions of initial states. In such a study, we showed that the expectation of equiprobable initial states is unrealistic and proposed to condition the probabilities of the initial values of the network nodes such that the outcomes would recapitulate the observed percentages of responding cells. This seminal work highlights the need to develop new methods to define the probability distributions of initial states based on experimental observations in an objective and formal way.

Openness and interoperability of models

The discrete dynamic models of sperm signaling reviewed here have been implemented and analyzed using specific in-house executed simulations and algorithms. Although the code is made available to other researchers upon request, it does not facilitate the modification and extension of the models. The difficulties of interoperability of models are not specific to sperm signaling, being a general challenge in systems biology. The Systems Biology Markup Language (SBML) was created to meet this challenge (Hucka et al., 2003). SBML is a community effort to maintain a standard software data format to describe models in systems biology. It is widely used to share models among a large community through the Biomodels repository of peer-reviewed models. A SBML Qualitative Models Package ("qual"), SBML qual (Chaouiya et al., 2013, 2015), is an extension of the SBML Level 3 standard (Keating et al., 2020), specifically tailored for the representation of multivalued qualitative models of biological networks. SBML qual is supported by several software applications dedicated to qualitative models, and it allows the exchange of these models among a number of complementary software tools, thus facilitating the analysis of models and potentiating collaboration among different groups. For example, analyzing a model with asynchronous dynamics or stochastic kinetics with distinct transition-specific rates could be readily performed with software tools such as GinSIM (Naldi et al., 2009) or MaBoSS (Stoll et al., 2017), respectively, if the models were available in SBML qual. It would be a worthy effort that the discrete dynamics models reviewed here be specified in SBML qual and shared through Biomodels database as this opens the analysis of sperm signaling to the variety of software tools to analyze qualitative models.

Enhancing sperm physiology models through artificial intelligence (AI) and machine learning (ML)

Network topology, regulatory interactions, and parameter values in the models we reviewed are manually curated through extensive literature reviews. AI and ML have been demonstrated to enhance model design, development, and optimization by improving parameter estimation and model selection techniques (Libbrecht & Noble, 2015; Merzbacher & Oyarzún, 2023). Additionally, AI-driven network analysis is instrumental in reconstructing and analyzing regulatory networks, inferring causal relationships crucial for

understanding biological pathways (Hill et al., 2016; Zito et al., 2023). These methodologies can enhance the modeling of sperm physiology, facilitating the discovery of regulatory interactions critical to fertilization, optimizing models to reproduce experimental results accurately and selecting the most suitable models to explain a determined phenomenon. Unlike traditional approaches that mostly use biological data as a framework for designing or validating models, AI and ML can detect patterns and integrate diverse biological data, enabling comprehensive modeling of complex systems (Merzbacher & Oyarzún, 2023). Incorporating data patterns and features into sperm physiology models could be essential for personalized medicine, aiding in the design of tailored fertility treatments and contraceptives, and have broad implications for biotechnology and pharmaceutical research in sperm physiology. The promise of AI and ML is, however, absolutely dependent on the availability of massive amounts of experimental data, an inevitable requirement for algorithm training.

Integration of ion-signaling models with models of sperm swimming

The biomechanics and physics of sperm swimming and chemotaxis have been the object of intense modeling studies (for sperm swimming, see Cass & Bloomfield-Gadêlha, 2023; Elgeti et al., 2010; Fuchter & Bloomfield-Gadêlha, 2023; Ishimoto et al., 2023; Q.-Y. Liu et al., 2020; Maggi et al., 2023; Nassir et al., 2022; Omori & Ishikawa, 2019; Smith et al., 2009; Tian & Wang, 2021; for chemotaxis, see Abdelgalil et al., 2022; Friedrich & Jülicher, 2007; Kromer et al., 2018; Lange & Friedrich, 2021; Naruse & Matsumoto, 2021; Ramírez-Gómez et al., 2020). These aspects constitute a complementary field that has been covered in other reviews (Alvarez et al., 2014; Gaffney et al., 2021; Guerrero et al., 2011, 2020; Ishimoto & Gaffney, 2015) and therefore not addressed here. Some modeling attempts to couple a reduced set of components of the sperm Ca^{2+}-signaling toolkit with the flagellar mechanics of mice sperm have been made (Carichino & Olson, 2018; Olson et al., 2011), rather than just making an abstract phenomenological description of $[Ca^{2+}]_i$. However, integrative attempts at whole-system modeling that couple full signaling networks and swimming mechanics, or that incorporate the phosphorylation networks that regulate and integrate ion fluxes during capacitation and the AR within a quantitative framework, have not yet been reported. The time is ripe for such holistic systems approaches.

The space-time encounter: Going beyond 1-dimensional models

In this presentation, we have dealt exclusively with time-dependent processes without considering spatial aspects. In fact, some studies, like the ones that only characterize the connectivity patterns in large networks (Bernabò et al., 2010; Bernabo et al., 2013; Bernabò et al., 2011; Bernabò et al., 2015; Bernabò et al., 2016; Bernabò, Mattioli, et al., 2015; Bernabò, Saponaro, et al., 2011) collapse all dynamics to provide a coarse-grained insight into how signals spread in a network in terms of causal chains of arbitrary-size steps. Moreover, ODE models (Prelle et al., 2022; Priego-Espinosa et al., 2020) are based on the well-mixed assumption (see Box 1), making explicit space or multiple-compartment considerations unfeasible.

The possibility of also introducing the system's dynamical behavior across space is enticing and much more realistic. This is the case of any of the formalisms we have addressed; examples would be the passage from systems of ordinary differential equations to partial differential equations, or the modeling of space/time discrete networks, cellular automata, or coupled map lattices (reviewed in Cowan et al., 2012; B. N. Kholodenko, 2006; Smart & Zilman, 2023). Such strategies would open the way for considering the extended dynamics along the flagellum or the axoneme/head coupling, to name a few.

Even though some models have addressed some sense of spatial dynamics by either proposing partial differential equations that consider the sperm as a 1-dimensional long compartment (Olson et al., 2010) or by applying delayed-dynamics equations to model the neck-to-principal-piece dynamics (Aguado-García et al., 2021), the spatial treatments of all the modeled elements do not account for any of the bi- or quadrilateral arrangements of signaling proteins recently observed along the sperm tail (Chung et al., 2014, 2017; Hwang et al., 2019; Zhao et al., 2022). The insights of mathematical modeling research in other types of cells could serve as inspiration to explore these fascinating features and their possible impact on signal compartmentalization into nanodomains (Giese et al., 2018; Hernández Mesa et al., 2022; Neves et al., 2008; Tadross et al., 2013), Ca^{2+} propagation through intracellular nonhomogeneous media (Keizer et al., 1998; Solovey & Dawson, 2010), and diffusion of second messengers inside complex cell geometries (Cugno et al., 2019), among others.

Final remarks

In this review, we recapitulated several models of sperm ion transport dynamics taking into account simplified versions of the signaling pathways involved in sperm swimming regulation, capacitation and acrosome reaction. These models revealed new possible and testable roles for certain ion channels, the significant impact of intracellular molecular heterogeneity in capacitation and the acrosome reaction, and the influence of intracellular and acrosomal pH on acrosomal exocytosis. The review addresses research with a complex systems perspective, where the multifactorial integration of the contributions of a variety of components into a collective, global behavior of the system as a whole is the goal. Several names have been attached to this program, starting from mathematical biology to systems biology or quantitative biology. Component connectivity and interdependence are starting points, and the introduction of dynamics is the crucial element for understanding

functionality. We hope we have managed to communicate the potential of the mathematization of the processes under study, this opens the possibility of prediction. We have emphasized that there is a diversity of formalisms available for this mathematization and that the choice, which can be multiple, is conditioned by the nature and aspects of the processes under consideration. Following the dynamics of entire configurations can reveal local behaviors otherwise unattainable due to restrictions and insufficient information. The available tools, be it theoretical, mathematical, or computational are in constant development; for example, the impressive advances in AI and ML are already impacting our capacity to integrate complex biological systems such as sperm to begin to fully understand quantitatively how signaling pathways translate into behavior. We foresee an auspicious future where the novel modeling tools at our disposal will expand our capacity to deepen our comprehension of sperm and cell physiology.

AUTHOR CONTRIBUTIONS

Daniel Priego Espinosa: Software; Investigation; Formal analysis; Writing — original draft; Methodology; Writing — review & editing; Data curation; Visualization. **Jesús Espinal-Enríquez**: Software; Investigation; Formal analysis; Writing — original draft; Writing — review & editing; Methodology; Data curation; Visualization. **Andrés Aldana**: Software; Investigation; Formal analysis; Writing — original draft; Methodology; Data curation; Writing — review & editing; Visualization. **Maximino Aldana**: Software; Formal analysis; Writing — original draft; Methodology; Supervision; Writing — review & editing. **Gustavo Martínez-Mekler**: Conceptualization; Formal analysis; Writing — original draft; Supervision; Resources; Writing — review & editing. **Jorge Carneiro**: Conceptualization; Investigation; Formal analysis; Writing — original draft; Writing — review & editing; Visualization; Supervision; Methodology. **Alberto Darszon**: Conceptualization; Investigation; Funding acquisition; Project administration; Writing — original draft; Writing — review & editing; Supervision; Resources; Validation.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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