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Is the idea of a fast block to polyspermy based on artifact?

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ABSTRACT

This purpose of this review is to look at the experimental evidence, both kinetic and electrophysiological, that led to the hypothesis of a fast electrical block to polyspermy in sea urchin eggs. The idea of a fast partial block, forwarded in the 1950's, that would reduce the receptivity of the egg surface by 1/20th following its interaction with the fertilizing spermatozoon, was based on experiments that treated fertilization as a first order chemical reaction. Here, I outline the criticisms of the Rothschild theory and demonstrate that the hypothesis of a fast partial block to polyspermy is unfounded. Notwithstanding, it was suggested in the 1970's that the membrane depolarization, induced by the fertilizing spermatozoon, prevented the interaction of supernumerary spermatozoa, the fast electrical block to polyspermy. While trans-membrane voltage recording has permitted a better understanding of the sequence of events occurring at fertilization, there is no evidence that depolarization prevents the interaction of supernumerary spermatozoa. Sperm entry is prevented at positive and negative potentials, in the voltage clamp configuration, however this is an artifact caused by the currents injected into the egg employed to hold the voltage constant in a non-physiological range. At permissive voltages, around −20 mV, where the current required to hold the voltage is minimal, only one spermatozoon normally enters the egg. Thus, irrespective of the egg voltage, the fertilizing spermatozoon is, in any case, attached to a privileged interaction site that permits entry and distinguishes it from supernumerary spermatozoa. Competence for monospermy is acquired during oocyte maturation and data on cortical organization in echinoderm eggs points to the actin filament system for regulating sperm entry.

1. Introduction

Polyspermy is to be avoided. If more than one sperm nucleus interacts with the female nucleus abnormal cleavage usually occurs and the embryo dies. There are two prevailing lines of thought as to how deuterostome eggs "resist polyspermy". First, is that the egg has evolved mechanisms to actively repel supernumerary spermatozoa [1–4], the second is that sperm–egg interactions in nature are finely regulated by behavioral, structural and molecular mechanisms to ensure monospermy [5,6].

Marine invertebrate eggs have been the model of choice for centuries owing to their abundance and amenability to laboratory conditions and manipulations, and the experimental animal par excellence is of course the sea urchin, favoured by scientists of note such as Hertwig, Boveri and Herbst [7]. Images of sea urchin eggs immersed in myriads of spermatozoa in a laboratory setting triggered the concept that fertilization, at least in these animals, was a haphazard "free for all", analogous to a first order chemical reaction and that the first spermatozoon that successfully interacted with the egg triggered membrane mechanisms that blocked the entry of other spermatozoa [8]. This change was suggested to be composed of a slow permanent block and a faster partial block and in the 1970's it was proposed that the fast block was electrical [9].

The purpose of this paper is to review the experimental evidence, both historical and recent, leading to the hypothesis of a fast electrical block to polyspermy, highlighting the criticisms, both in logic and in fact, and to propose an alternative reason as to how eggs ensure monospermy. The traditional terminology of a sea urchin egg is used to indicate a meiotically mature cell, and a sea urchin oocyte to indicate a meiotically immature germinal vescicle stage cell.

2. Fertilization studies in the laboratory

"If the condition of the eggs is not taken into account, the results obtained by the use of sub-normal eggs in experiments may be due wholly or in part to the poor physiological condition of the eggs. Thus, the failure of sea-urchins eggs that are freed of their jelly, fully to

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separate their vitelline membrane after fertilization, as they normally do, does not mean that the experimental removal of jelly renders membrane separation impossible but only that the eggs are in a bad condition brought about by injurious action of the agent employed to remove the jelly". Quote from Ernest E. Just [10].

2.1. Materials and methods

For those less familiar with echinoderm embryology, below is a brief description on how the gametes are procured and used in the laboratory. First, animals are removed from their natural environment by divers in an ad hoc fashion, not taking into account their breeding patterns or indeed their sexual maturity. Both, during transit, and in the laboratory, the sea urchins are held in tanks for varying times, often at temperatures below that of the environment. The sea urchins may be induced to spawn by injecting 0.5 M K Cl (an electrophysiological experiment in its own right) into their cavities, or their gonads removed by manual dissection. In both methods the gametes are exposed to visceral fluids which as pointed out by Ernest Just [10] may also influence the state of the gametes. The egg jelly is considered to be an impediment by some scientists and therefore is removed either by exposure to acid seawater, repeatedly rinsing through meshes, or leaving the eggs in sea water, where, over time, the jelly expands and eventually dissociates from the vitelline membrane [11]. Finally, for electrophysiological experiments, some authors prefer for the eggs to attach electrostatically to plastic dishes or to poly L-lysine coated dishes. In Naples, sub-optimal specimens of *Paracentrotus lividus* have dark brown ovaries, the eggs vary in size and there are more than 1% germinal vesicle oocytes present, while optimal specimens have large red ovaries with homogeneous egg size and less than 1% germinal vesicle stage oocytes [12]. It is well known that meiotic maturity is not necessarily correlated with cytoplasmic maturity [13] and in fact Runnstrom and Monne [14] have shown that underipe, ripe and overripe sea urchin eggs have very different membrane properties, while Borei [15], by measuring oxygen consumption, showed that sea urchin eggs age rapidly when extracted from their ovaries.

2.2. The history and origin of the fast block hypothesis

"*Any worker possessing only mediocre powers of observation, therefore, should be able to prove to his own satisfaction that eggs, of these three forms at least* (referring to three species of Sea urchin), *separate membranes, beginning at the point of sperm entry*" Quote from Ernest E. Just [16]. In 1919, Just [17] had previously quoted "*before the actual elevation of the fertilization membrane, some cortical change beginning at the point of sperm entry sweeps over the egg, immunizing it to other sperm*", while in 1939 Just, in his landmark book [10], suggests this change may be attributable to nerve conduction, "*because among animal cells it is the most highly excitable and the most rapidly conducting*".

Sperm–egg kinetics and in particular why only one spermatozoon enters an egg requires a model amenable to observation in the laboratory. Molecular tools are of little help in elucidating the kinetics of fertilization owing to the number and rapidity of events occurring in these first few moments. The sea urchin egg, owing to its availability and transparency, has been invaluable in studying these initial events, however it has also been the cause of much controversy. The question posed is: "does the fertilizing spermatozoon block the entry of supernumerary spermatozoa by inducing a change in egg physiology, or are the supernumerary spermatozoa attached to sites that do not permit entry" (Fig. 1).

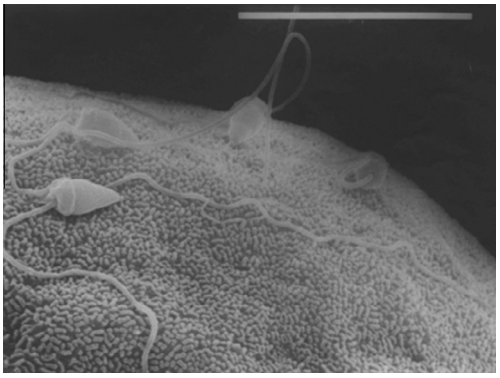

Fig. 1. The surface of a jelly-free sea urchin egg at the scanning electron microscope. Is the fertilizing spermatozoon attached to a privileged entry site while the supernumerary spermatozoa are attached to abortive sites that do not permit entry?

2.3. Kinetic experiments of Lord Rothschild

Students of fertilization should delve into the works of Rothschild and colleagues in the 1940–50's who studied sperm–egg interaction in sea urchins at various concentrations and conditions and came up with the concept that the fertilizing spermatozoon induced a fast, yet partial, change in the egg surface that preceded the cortical reaction and that reduced sperm receptivity by 1/20th.

The first paper was observational. "*It has of course been known since the nineteenth century that the surface of the sea urchin egg changes at fertilization, and the method of observing this change, whether with polarized light, dark ground illumination or ordinary light...*" [18]. The authors concluded that there was a change in cortical structure, seen in dark field illumination as a scattering of light, of the sea urchin egg *Psammechinus miliaris* that covers the egg in about 20 s at 18 °C. "*At the site of spermatozoon entry, there is a localized and transient decrease in light scattering, the elevation of a fertilization cone and it is here that the fertilization membrane starts to elevate*". By treating the spermatozoa as an "assembly of gas molecules" and considering the mean translator speed of spermatozoa to be 190 μ/sec, the number of sperm egg collisions at 10^5/ml would be 1.6, at 10^6/ml would be 16, and at 10^7/ml would be 160. Using immature oocytes, "*which do not have any resistance to polyspermy and sperm entry may be easily counted by counting the number of blebs*" (fertilization cone like structures) the authors demonstrated that from an expected 4500 collisions at a sperm density of 10^7/ml, in a 5 min period, less than 100 actually entered. Rothschild and Swann [18] concluded "*only a fraction of the spermatozoa which collide with the egg surface are able to initiate activation*" and "*attachment of the spermatozoon to an egg is not followed by fertilization unless there exists a particular orientation on a molecular scale, between the egg and sperm surfaces, and provided there has been no previous interaction between spermatozoa and Gynogamone II*" (an agglutinating substance released from eggs).

In the second series of experiments, Lord Rothschild devised an ingenious kinetic experiment measuring the probability of successful sperm–egg collisions by immersing eggs in sperm suspensions of known density for known, but varying, periods of time and later counting the proportions of fertilized and unfertilized eggs. By treating the fertilization reaction as a first order chemical reaction, Rothschild and Swann [19,20] found that the fraction of monospermic eggs in *P. miliaris* increased in time according to the relationship

$M(t) = 1 - e^{-\alpha t}$ below sperm densities of 3×10^6/ml.

The relationship is similar for polyspermic eggs at densities between 7×10^7/ml and 3×10^8/ml giving a rate of appearance of polyspermic eggs as α^1.

Since α^1 (the refertilization rate) was found to be much less than α (the monospermic rate), it was concluded that a rapidly acting partial block reduced the probability of successful reactions after the first occurred. In order to create a $T = 0$ for fertilization, Lord Rothschild used a sperm concentration of 10^8/ml, which for anybody familiar with sea urchin embryology is 10–100 times the concentration used in normal laboratory experiments, probably well over 1000 the concentration found in nature (see later), and slightly less than that extracted directly from the gonads.

Rothschild [19,20] points out the drawbacks of his own experiments:

"*Since α^1 is the rate of increase of polyspermic eggs, it is perforce an underestimate of the successful collision rate because it does not take into account the actual number of sperm per polyspermic egg. In addition, the non linear dependence of α on n may be due to sperm-sperm interactions at higher densities, while we have not taken into account chemotaxis from egg exuded components, the presence of the jelly or the declining fertilizing capacity of spermatozoa in time.*"

The Rothschild hypothesis has fundamental errors in logic:

1) Fertilization is not a first order chemical reaction and spermatozoa are not analogous to gas molecules.
2) The hypothesis does not consider the possibility that there could be a limited number of sites on the egg surface through which spermatozoa may enter.
3) It assumes all spermatozoa are capable of activating or penetrating the cell. This is not true. Only competent spermatozoa that encounter and respond to the correct sequence of triggering events as they progress through the egg investments are successful.

3. Electrical events at fertilization

"*The basis and the control of any experiment is the perfectly normal egg; the worker must know therefore what is a good egg*". Quote from Ernest E. Just [10].

Ionic homeostasis is paramount to life and is controlled in both gametes and somatic cells by transmembrane proteins, ion channels and transporters. Ion transport through channels is passive and these proteins may be voltage-gated or ligand-gated, while transporters, often called "pumps" require energy to pump solutes across membranes against their electrochemical gradients. Primary transporters use ATP directly, while secondary transporters, also called co-transporters, use ATP-established ion gradients for Na^+ or H^+ as an energy source [21]. Gametes possess a variety of ion channels and transporters that are essential for their function [22] and sea urchin oocytes and eggs contain Ca^{2+}, Na^+ and K^+ channels [23,24]. It has been known for over 60 years that ion fluxes are involved in the process of egg activation [25–27].

3.1. What happens electrically at fertilization

The first event of activation in sea urchin eggs is a membrane depolarization accompanied by a decrease in resistance and increase in membrane noise (Fig. 2) [28]. This initial event, also observed in other deuterostome oocytes (see review [22]), is followed some 13 s later, at 22 °C, by a slower longer lasting depolarization called the fertilization potential. Using the traditional two electrode voltage clamp technique, the current generated at fertilization in sea urchin eggs, at a clamp voltage of −20 mV, was found to be biphasic and in fact the mirror image of the voltage response [29].

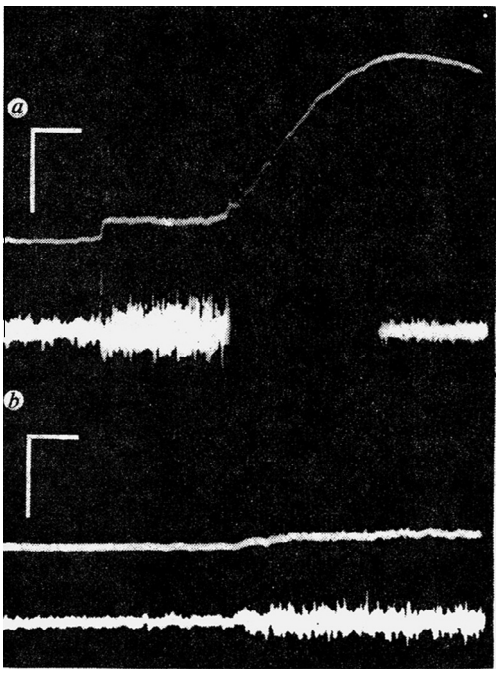

Fig. 2. The voltage change at fertilization in the sea urchin egg. (A) The bell-shaped fertilization potential is preceded by about 11 s by the step depolarization at 25 °C. The time between the two is called the latent period. The trace below shows the increase in voltage noise which, together with the change in resistance, led to the calculation of the elementary conductance change to be 33 pS. (B) The events at 5 °C showing the increase in length of the latent period. The horizontal bars represent 5 s, the vertical bar represents 6 mV (upper) and 1 mV (lower).

The elementary conductance event underlying the step depolarization was calculated by noise analysis to be 33 pS and, by analogy with other known ion channels, whose reversal potential suggested a relatively non-ion specific channel, the fertilization channel. Although it was not possible to patch clamp the sea urchin egg, due to the tightly adhering vitelline coat, direct measurements in the ascidian, gave values of up to 400 pS for the fertilization channel [30]. Using the whole cell voltage clamp technique the ascidian fertilization current was found to be inward and bell-shaped reaching a peak of 1 nA after about 30 s [31,32]. This current, may involve the movement of 10^{10} ions which, if localized to the sub-cortical cytoplasm could increase the cation concentration to 10 mM [6]. Thus the fertilization current alone may drastically change the sub-cortical concentration of free cations, before ion pumps are able to re-establish pre-fertilization values, priming or modulating the subsequent intracellular Ca^{2+} release mechanisms, which could be responsible for the cortical Ca^{2+}flash in sea urchin eggs (see [81]). It has been estimated that a spermatozoon triggers 200–2000 fertilization channels around the point of sperm–egg fusion [22,32]. In the sea urchin the fertilization current is about −500 pA, while the conductance increases from 20 to 40 nS and the reversal potential is +10 mV again suggesting it is nonspecific for ions [33]. Other types of ion channel, such as Ca^{2+} and Na^+ channels, may be secondarily activated at fertilization in sea urchin eggs, owing to their voltage dependence, however the reversal potentials for these currents are much higher than +0 mV.

3.2. Monitoring the kinetics of sperm egg interaction by electrical recording

The size and transparency of eggs from many sea urchins make it extremely easy to observe morphological events and correlate

these with electrical measurements. Many spermatozoa attach to the surface of the jelly-free egg, however it is the fertilizing spermatozoon (by no means the first that arrives) [12], that distinguishes itself by gyrating around its point of attachment. About 3secs later the step depolarization event is generated (Fig. 3), with no change in the morphology of the egg surface or sperm behavior, until 10 s later when the larger fertilization potential starts. At this time, the fertilizing spermatozoon stops gyrating, its tail stiffens and the fertilization membrane starts to elevate (the beginning of cortical granule exocytosis). The head of the spermatozoon is seen to be absorbed by the fertilization cone and the cortical reaction is completed during the falling phase of the fertilization potential [12]. The period between the two electrical events correlates temporarily with what has been termed the latent period [35]. The delay between the two electrical events is temperature dependant [28] and may be varied in length by treating eggs with cytochalasin drugs that interfere with microfilament physiology [36].

Immature germinal vesicle stage oocytes lack cortical granules and are known to be polyspermic under conditions where mature eggs are monospermic [18]. Spermatozoa that successfully penetrate immature oocytes generate a step depolarization, as in eggs, and may also be identified by their gyrating behavior which lasts up to 60 s before they stiffen and are engulfed by cytoplasmic blebs (Fig. 3 [37,38]). There are two types of unsuccessful, attached, but non-penetrating spermatozoa. Spermatozoa that do not generate an electrical step event, do not stiffen and continue gyrating for several minutes and others that generate an additive step depolarization that subsequently turns off [37,39].

When sea urchin eggs were voltage clamped at +15 mV with two intracellular electrodes and sperm added, spermatozoa attached to the surface but were unable to induce activation [40]. Briefly repolarizing the cell to −60 mV, for a 30–60 ms time window, was sufficient for sperm–egg interaction to proceed and for successful fertilization. The authors showed that the period between the reversal of the voltage clamp and the start of the activation current (the latent period) was highly variable and ranged between 13 and 25 s. By voltage clamping sea urchin eggs and then fixing them for electron microscopy at varying times after insemination, Longo and colleagues showed that the first electrical event was detected 2 s after attachment, with gamete fusion occurring at 7 s and cortical exocytosis starting at 9 s. The fertilization potential was detected at 13 s [41]. Using live sea urchin eggs, loaded with a fluorescent dye, cytoplasmic continuity between sperm and egg was determined when the dye flowed from the egg into the spermatozoon [42]. By inseminating voltage clamped (−20 mV) dye-loaded eggs, Hinkley and colleagues inferred that cell–cell continuity occurred at 4–8 s after the initial electrical event. Again, using the traditional voltage clamp configuration, Lynn and colleagues [43] showed that while sperm entry was blocked at holding potentials of −75 mV or at +20 mV, at clamp potentials of +17 mV to −20 mV a characteristic biphasic fertilization current was generated associated with normal sperm entry. At clamp potentials more negative than −20 mV, fertilization currents were anomalous, including currents which abruptly reversed, similar to the voltage steps that turn off seen in sea urchin oocytes [37,39], and sperm did not enter or activate the egg.

3.3. Experiments that led to the proposal for a fast electrical block to polyspermy

3.3.1. Experiments in current clamp conditions

In 1976, Jaffe [9], using de-jellied sea urchin eggs attached electrostatically to plastic falcon dishes, distinguished two types of electrical response at fertilization. Under current clamp conditions, 8 eggs that generated a fertilization potential more positive than 0 mV following insemination were monospermic, while of 13 eggs that generated a fertilization potential less positive than −10 mV, 6 were monospermic and 7 were polyspermic. "*These results suggest that the entry of extra sperm is prevented by the more positive-going activation potential*" [9]. The author went on to show that eggs clamped at +5 mV, by current injection, could not be activated when sperm were added to the chamber. However, the eggs in this inappropriate configuration of current clamp were held positive by uncontrolled and unmeasured amounts of positive current. Jaffe [9] acknowledged that incapacity to block polyspermy might be due to egg deterioration. It is common knowledge that some egg batches are prone to polyspermy [20]), confirmed by both Jaffe [9] and Dale and DeFelice [39], who also demonstrated that the

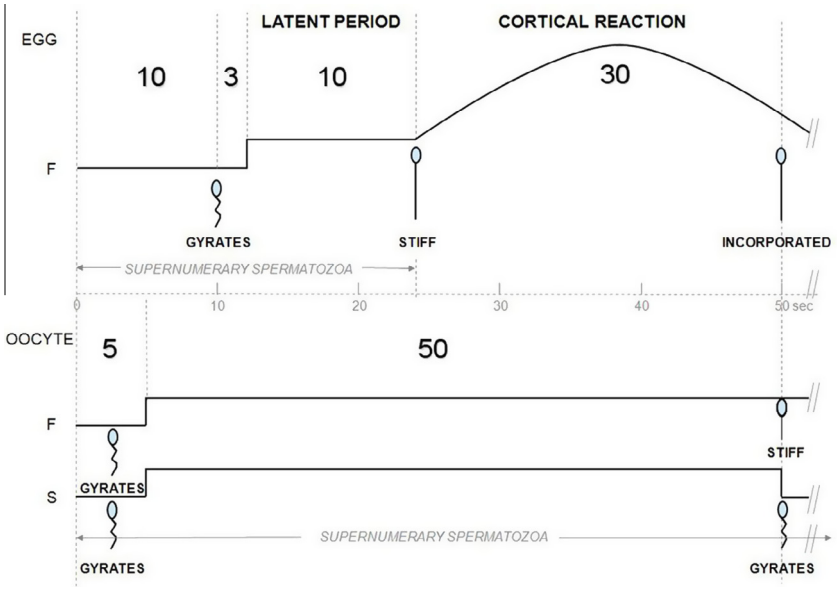

Fig. 3. A schematic diagram of the voltage changes in a sea urchin egg and a sea urchin oocyte following insemination showing the behaviour of the fertilizing spermatozoon. Note that in both cases supernumerary spermatozoa attach to the egg/oocyte surface before and after the step event.

impaled egg behaved as the surroundings eggs. i.e., that polyspermy was not induced by the experimental procedure.

3.3.2. Experiments in the voltage clamp configuration

Using a conventional voltage clamp configuration, Shen and Steinhardt [40] showed that sea urchin eggs clamped at a membrane potential of +15 mV and then inseminated cannot be activated, unless the potential is briefly re-polarized for 30–60 ms to a negative potential. Eggs voltage clamped at 0 to +13 mV could however be activated by spermatozoa. Shen and Steinhardt [40] conclude "*we were somewhat surprised to observe a low rate of polyspermy after the application of a window of negativity. Despite the presence of numerous sperm, all presumably blocked at the same point, nearly all the fertilizations were monospermic*". In a more detailed voltage clamp study, Lynn and colleagues [43] demonstrated that spermatozoa were prevented from successfully interacting with the eggs, either at clamped voltages more positive than +17 mV, or more negative than −75 mV, however successful activation occurred at intermediate clamp voltages of −20 to +17 mV (Fig. 3). These authors conclude "*that the mechanism of inhibition differs in the two Vm ranges*" i.e., +17 mV and −75 mV) while "*Depolarization beyond −25 mV permits sperm entry to proceed*". Again the authors note that "*As many as 5–25 sperm may attach before a successful encounter occurs that results in an electrophysiological response*". In summary, Lynn and colleagues [43] proposed that the positive voltage block is, in effect, the fast electrical block to polyspermy, while the block to sperm entry at negative voltages demonstrates that a positive shift in membrane potential is required for sperm entry.

3.4. Arguments against the idea of a fast electrical block

3.4.1. Kinetics

In the voltage clamp experiments of Shen and Steinhardt [40], the authors elegantly demonstrated that while positive voltages blocked the entry of all the spermatozoa attached to the surface of the egg, a negative re-polarization permitted the entry of only one spermatozoon despite "*the presence of numerous sperm*" (Fig. 4). In an analogous situation, using a fluorescent dye to indicate cell–cell fusion, Hinkley et al. [42] showed that in sea urchin eggs voltage clamped at −20 mV, only one spermatozoon fused with the egg. In the Lynn study, [43], clamp voltages of −20 mV to +17 mV, permitted normal sperm entry associated with a typical biphasic fertilization current. It is apparent from the above experiments, that the fertilizing spermatozoon is intrinsically different to the supernumerary spermatozoa or located at a preferential entry site; irrespective of any membrane voltage change.

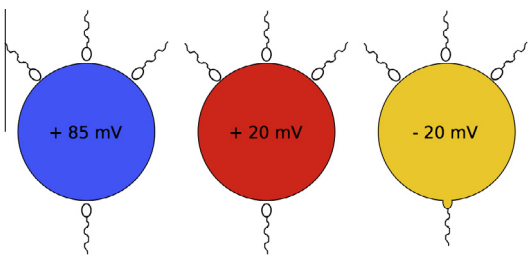

Fig. 4. A schematic representation of voltage clamp experiments in the sea urchin egg. Holding the egg at extreme negative values (blue) or extreme positive values (red) inhibits interaction of all spermatozoa owing to the non-physiological currents generated. Lowering the voltage to −20 mV (yellow), and consequently reducing the amount of current injected, permits sperm fusion, but for only one – the pre-determined spermatozoon!

3.4.2. Does the voltage clamp configuration- not voltage per se-block sperm–egg interactions?

In order to measure directly the ion currents across a biological membrane it is necessary to voltage clamp the membrane, either by the whole cell clamp technique or by using two intracellular electrodes, one to measure the current, the other to inject current to move the transmembrane voltage to a particular value. Voltage clamp was traditionally designed for nerve and muscle cells or in any case cells that are, at most, exposed to variations in voltage or to the application of drugs. The sea urchin egg is nearly one million times the volume of the spermatozoon. The spermatozoon undergoes a sequential series of physiological changes as it penetrates the egg investments triggered by a series of molecular interactions that leads to physiological competence [6]. Clamping this large cell at a given voltage, particularly at a voltage extraneous to the cells natural resting potential, implicates injecting potentially large amounts of current into the egg. This raises the question as to whether the non-physiological currents generated across the plasma membrane, by nature of holding the voltage steady in the voltage clamp configuration, interferes with sperm–egg interaction. In other words the observed block to sperm entry in voltage clamped eggs is artifactual – due to the un-physiological currents injected through the microelectrodes. Clamping eggs at voltages distant from their natural resting potentials i.e., +20 mV or −80 mV requires large amounts of current and blocks sperm entry due to a molecular re-organization of the egg plasma membrane (frying), while clamping around the natural resting potential, or at least in a range where little current is required to hold the membrane steady, i.e., −20 mV to +15 mV, allows sperm entry (see Fig. 4). Depolarization of the egg membrane at activation has not evolved to prevent polyspermy, but rather it is an integral part of the activation process, perhaps responsible for the cortical Ca^{2+} flash (see [81]).

4. Monospermic fertilization

"*Descriptive embryology built upon laboratory observations stands only if one can assume that the stages observed represent faithful reproductions of those occurring in nature*..." "E.E. Just [10].

4.1. Sperm–egg ratios in situ

When dealing with sea urchin gametes in the laboratory a sperm–egg ratio that leads to 100% fertilization at time 0, with minimum rates of polyspermy is often selected ($\geqslant 10^6$/ml), however data collected from natural spawnings show a much lower fertilization rate and great variability in the proportion of eggs fertilized in nature. Fertilization success in nature depends on spawning behavior of the organisms, population size, current velocity, egg size, sperm swimming capacity and many other factors. The consensus from field studies is that fertilization success in free-spawning benthic organisms can be less than 1% [44] and in any case is highly variable, ranging from 1% to 95%. Thus, in the environment, sperm–egg collisions are rare, sperm concentration may be extremely low below ($\leqslant 10^4$/ml) and the availability of sperm may affect female reproductive success (see review on Sperm limitation [45]). If, under natural conditions, sperm–egg collisions are indeed low, perhaps we should re-consider some of our ideas on the evolution of sexual dimorphism.

4.2. Fertilization is not a first order chemical reaction

At spawning sea urchin sperm are exposed to the higher pH of the sea water which leads to the activation of a Na^+/H^+ exchanger, a Dynein ATPase and increased motility [46]. Sperm behavior is then

further modulated by factors released from the extracellular coats of the eggs [47]. Speract and Resact (sperm-activating peptides) have been isolated from the jelly layer and have been shown to stimulate sperm motility and respiration [48–51].

On reaching the egg the spermatozoa must interact with the various egg investments. As the spermatozoon passes through these layers its physiology changes and successive passage is dependant on these changes [34]. The first investment in the sea urchin is the jelly layer, which since it may be removed without impeding fertilization, has often been considered an accessory rather than an integral part of the egg. Little is known about the structure of the jelly layer in situ. It is composed of high molecular weight fucose-sulfate-rich glycoconjugates and seems to have a slightly lower pH than the surrounding sea water [52], which may play a role in sperm progression and activation. There is no data on whether the jelly layer is radially organized, however a jelly canal at the animal pole has been identified and suggested to be a preferential sperm entry site analogous to a micropyle [53–55]. There is little information on the structure of the jelly layer in sea urchin eggs, however in amphibians eggs the jelly is a fibrillar matrix of high molecular weight glycoproteins, interspersed with globular proteins of lower molecular weight [56], and an unusual hypertonic milieu of 70 mM Na^+, 30 mM K^+, 6 mM Ca^{2+} and 7 mM Mg^{2+} [57].

The kinetics of fertilization also depends on the age of the gametes. Spermatozoa deplete energy resources quite rapidly [45], while eggs need to be fertilized at a precise moment in time. If eggs are underipe or overripe they invariably become polyspermic. The jelly layer in aged sea urchin eggs spontaneously dissolves, while many activation events are initiated precociously for example the dissolution of cortical granules. Polyspermy may be induced in mature eggs by exposure to a wide collection of physical and chemical agents, with the most commonly used being Nicotine. Brief exposure to this alkaloid increases the receptivity of the sea urchin egg to spermatozoa, possibly by changing the intracellular pH of the egg cortex [58,59].

4.3. Polarized sperm entry, activation and actin

Eggs are polarized cells, the animal pole marking the position of the meiotic plate, with organelles distributed in a gradient towards the vegetal pole. In most animals, the spermatozoon enters the egg at a preferential site or area, for example in ascidians they enter at the vegetal pole, in anurans at the animal pole. The most marked example for a pre-determined site is to be found in the insects and teleosts where the egg lacks cortical granules and is protected by a pre-formed impenetrable protective coat, the chorion. Here spermatozoa enter the egg through a preformed entry site the micropyle [5], where a sperm attractant, a glycol-protein, has been identified responsible for guiding the spermatozoa to the micropyle [60]. Egg activation events are also polarized, the first being the release of calcium from intracellular stores which traverses the egg to the antipode in a wave [24,61]. In jellyfish and Xenopus eggs the calcium wave arises at the animal pole, the sperm entry site [62–64], while in ascidian and nemerteans eggs the wave initiates at the vegetal pole, the site of sperm entry [65,66]. In fish eggs the calcium wave originates at the sperm entry site at the animal pole [67–69]. If we consider that all animals are in fact models for others and we know that in mammals and echinoderms the calcium wave initiates at the region of sperm entry [70–74] it would be interesting to re-evaluate whether there are preferential sperm entry sites in these common deuterostomes.

Cortical organization in eggs changes with maturity and involves the length and density of microvilli, the location of cortical granules [37,75,76] and the priming of the intracellular Ca^{2+} release mechanism [74]. All events tightly controlled by the actin cortical cytoskeleton [77]. That actin microfilaments are involved in sperm entry has been known for decades and recently, by using confocal and digitally enhanced video microscopy in the starfish egg, it has been shown that altering the organization of cortical actin filaments renders the egg more receptive to spermatozoa and induces polyspermy [78–80]. In monospermic fertilization in the starfish, the successful spermatozoa distinguishes itself from supernumerary spermatozoa by tethering itself to a sub-cortical actin anchor that ensures rapid incorporation, while supernumerary spermatozoa attach to sites that lead to abortive sub-cortical events [78].

In this review, I have presented arguments to refute the idea of a fast electrical block to polyspermy, both based on the logic for treating sperm–egg interactions as a first order chemical reaction and the artifacts created by voltage clamping sea urchin eggs. The cortical flash in sea urchin eggs, that precedes the calcium wave (see [81]) by several seconds, could potentially change the cortical actin cytoskeleton and render the egg unreceptive to other spermatozoa [74], however it is implicit that the sperm causing this flash is, in any case, different to the others, or by virtue of its competence, or because it is attached to a privileged entry site; perhaps an actin-rich site, generated during cytoplasmic maturation, which may be located randomly over the egg surface with respect to the *A–V* axis.

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