SPERM-OOCYTE INTERACTION IN THE SEA-URCHIN

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SUMMARY

A study was made of the electrical and morphological changes in the sea-urchin oocyte during interaction with spermatozoa. The first event, a small step depolarization accompanied by a 20–40% decay in input resistance occurs within seconds of attachment. The evidence suggests that this electrical event is the result of sperm-oocyte fusion, and that the ion channels that lower the resistance across the oocyte-sperm complex are located in the sperm plasma membrane. This primary electrical event does not necessarily lead to sperm incorporation. A second, determinative, event occurs at 50 s, which leads to sperm entry and the formation of a cytoplasmic protrusion at the site of sperm entry. This second event probably results from the transfer of a soluble component from the spermatozoon into the oocyte cytoplasm, which leads to sperm incorporation and formation of the protrusion. The changes in the oocyte following insemination are compared with the events of egg activation.

INTRODUCTION

Many basic cell processes such as the mitotic cycle, differentiation and proliferation are regulated by ionic signals (Boynton, McKeehan & Whitfield, 1982). Changes in the concentration of intracellular ions may result from the release of ions from internal stores or from alterations in the permeability of the plasma membrane. Both of these mechanisms appear to be involved in the early events of fertilization (Dale, 1983).

In the sea-urchin the first indication of activation is a depolarization of the egg plasma membrane (Steinhardt, Lundin & Mazia, 1971; Ito & Yoshioka, 1973; Jaffe, 1976; Chambers & de Armendi, 1979). Subsequent studies have shown that this depolarization, called the fertilization potential, may be composed of at least two events. The initial depolarization is accompanied by an increase in membrane noise due to the activation of non-specific ionic channels (Dale, De Felice & Taglietti, 1978). There is no further change for several seconds, then a second, slower depolarization is initiated simultaneously with the breakdown of the cortical granules (Dale & de Santis, 1981). At about this time the fertilizing spermatozoon stops gyrating around its point of attachment (Dale & de Santis, 1981). In addition to these changes at the plasma membrane there is a release of Ca^{2+} from intracellular stores (Steinhardt, Zucker & Schatten, 1977), which starts at the point of spermatozoon attachment and spreads around the egg in a wave (Eisen, Reynolds, Wielands & Richart, 1983). This transient increase in free intracellular Ca^{2+} is thought to be a

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Key words: sea-urchin, sperm, oocyte, electrical events.
pre-requisite for subsequent events of activation, such as cortical granule breakdown (Epel, 1980; Whitaker & Steinhardt, 1982).

The early stages of sperm–egg interaction involve many structural changes; however, it is generally accepted that there are two major steps. First, the species specific recognition and binding of the two gametes; this is followed by the fusion of the sperm and egg plasma membranes. In order to fuse the sperm undergo a structural modification called the acrosome reaction, which results in the release of lytic enzymes and exposure of the fusogenic acrosomal tubule. There is some controversy over the site of the acrosome reaction. Some authors believe it occurs while the spermatozoon is penetrating the jelly layer, others have suggested it occurs following the attachment of the spermatozoon to the vitelline coat (see Dale & Monroy, 1981; Lopo, 1983, for discussion). It is not known at which stage of sperm–egg interaction the spermatozoon triggers the ionic events associated with egg activation.

Spermatozoa are also able to penetrate the immature germinal vesicle stage oocyte; however, little is known about the mechanism involved. Each sperm entry is marked by a step depolarization (De Felice & Dale, 1979) and the formation of a large cytoplasmic protrusion on the oocyte surface (Wilson, 1895); however, there is no cortical reaction (Runnström & Monné, 1945). The object of the present work was to study in more detail the physiological and morphological changes in the sea-urchin oocyte during interaction with spermatozoa and to compare these changes with the events of egg activation.

MATERIALS AND METHODS

Experiments were carried out on gametes of the sea-urchins Paracentrotus lividus and Sphaerechinus granularis collected from the Bay of Naples. Gametes were obtained by dissection. In the former species batches of eggs containing more than 1% oocytes were selected. Oocytes are recognized by their large clear nuclei (see Fig. 1). In the case of Sphaerechinus the gonads were teased apart to release the immature gametes. In the majority of cases oocytes and eggs were used with their jelly layers intact. Unless indicated otherwise, natural sea water (NSW) at ambient temperature (18°C–20°C) was used. Spermatozoa were kept in the 'dry state' until immediately before each experiment, when they were diluted with sea water.

Electrical recordings were made on oocytes on glass slides, to which they did not adhere. Insemination was carried out as described elsewhere (Dale, 1985). Glass microelectrodes filled with 1.8 M-potassium citrate and having 40–80 MΩ resistance were used for the intracellular recordings. Measurements of membrane resistance were made with two intracellular micro-electrodes: one to record voltage, the second to inject current via a limiting current resistor of 3 X 10⁶Ω. All recordings were stored on FM tape for subsequent analysis.

Gametes were fixed at room temperature in 1% glutaraldehyde in 77% sea water and post-fixed in 1% osmium tetroxide. The material was dehydrated in ethanol, embedded in Epon and sections were observed with a Philips 400 electron microscope. The artificial Mg-free sea water (Mg FSW) had the following composition: 575 mM-NaCl, 10 mM-KCl, 10 mM-CaCl₂, 2 mM-NaHCO₃. Quercetin (Sigma, St Louis) was made up in ethanol at 10 mM and the ionophore A23187 (Sigma, St Louis) in dimethyl sulphoxide at 2 mM.

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Fig. 1. A time sequence of sperm–oocyte interaction in the sea-urchin S. granularis in NSW at 18°C. Note that some of the attached spermatozoa do not induce cytoplasmic protrusions (arrows). Bar 20 μm.
Sperm-oocyte interaction in the sea-urchin

Fig. 1
RESULTS

Morphological events

A time sequence of sperm–oocyte interaction in the sea-urchin *S. granularis* at 18 °C is shown in Fig. 1. At moderate to high sperm densities (5 × 10^5 to 10^6/ml) spermatozoa attach to the oocyte surface within a few seconds of insemination; however, not all of the attached spermatozoa are capable of penetrating the oocyte. Each spermatozoon that penetrates the oocyte first gyrates around its point of attachment for 30–60 s and then becomes immotile with its tail extending perpendicular to the oocyte surface. At this time a protrusion of the oocyte surface grows and engulfs the sperm head and continues swelling for some 5 min. In contrast, unsuccessful spermatozoa continue gyrating for several minutes without inducing a 'bleb' and eventually break away from the oocyte or fall motionless against the surface. Bleb formation in oocytes appears to be analogous to the formation of the fertilization cone in the mature egg (Franklin, 1965). As reported previously, oocyte blebs are usually retracted 10–20 min after insemination (Hagström & Lönning, 1961; Runnström, 1963).

There have been many studies on the ultrastructure of sea-urchin eggs and oocytes (Runnström, 1963; Lönning, 1965; Franklin, 1965; Kidd, 1978; Longo, 1978; Tilney & Jaffe, 1980); however, we wish to point out several aspects of particular relevance to our present study. First, germinal vesicle stage oocytes have an extensive surface area due to the presence of long and numerous microvilli (Fig. 2A), whereas the mature egg has fewer and smaller microvilli (0.1–0.3 μm; Fig. 2C). A second difference is that the egg surface is covered by a fibrous extracellular coat, the vitelline coat (Fig. 2D), which is not obvious in the oocyte (Fig. 2B). The first response to insemination in the egg is the elevation of this coat caused by the disruption of the cortical granules (arrows, Fig. 2C). Morphologically similar granules are present in the oocyte; however, they are located deeper in the cytoplasm. The periphery of full-size oocytes (1–3 μm deep) is practically devoid of large organelles (Fig. 2A).

In order to correlate the morphological and physiological events of sperm entry into oocytes, samples of oocytes were fixed at various intervals after insemination. At the same time oocytes were selected for electrophysiological recording and inseminated under identical conditions of temperature and sperm density. The electrophysiological events will be dealt with in the next section; it is sufficient here to point out that the sperm-induced depolarizations occurred within 5 s of insemination. Under the conditions used many thousands of spermatozoa appeared to attach to the oocytes within seconds; however, the majority of semi-thin sections of material fixed at 5–20 s post-

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Fig. 2. Electron micrographs to show the surface structure of sea-urchin oocytes (A, B) and mature eggs (C, D). Note that the oocyte surface is bounded by frequent and long microvilli (A) and has an extensive cortical endoplasmic reticulum. The plasma membrane appears, for the most part, to be nude (B). In contrast, the egg has short infrequent microvilli (C), and the plasma membrane is covered by a fibrous vitelline coat (D). The cortical granules (arrows) are found throughout the cytoplasm in oocytes, whereas they become aligned beneath the plasma membrane in eggs. A, C, × 9660; B, D, × 46 000.
Sperm-oocyte interaction in the sea-urchin

Fig. 2
Fig. 3. Various stages of sperm incorporation in oocytes of *S. granularis* at 20°C. A. An acrosome reacted spermatozoon 50 s post-insemination. Note there is no change in the underlying cytoplasm of the oocyte. Cortical granules (arrow) are still present and the cortical endoplasmic reticulum is unaltered. B. At 2 min post-insemination the spermatozoon head has entered the oocyte and the cytoplasm has been raised around it in a protuberance. Again the underlying endoplasmic reticulum and cortical granules are apparently unaltered. C. At 5 min post-insemination the sperm nucleus has rotated 90° to the plane of entry and the protrusion has swollen into a large organelle-free bleb. A, × 21,000; B, × 9,600; C, × 12,600.

insemination were devoid of attached spermatozoa. Presumably, many sperm attachments are labile and the spermatozoa are dislodged during fixation. In semi-thin sections of material fixed at 20 s to 5 min post-insemination we usually observed
between 2 and 10 attached spermatozoa. In 200 sections, more than 95% of the attached spermatozoa had unreacted acrosomes. The first indication of acrosome-reacted attached spermatozoa was at 20 s; an example at 50 s is shown in Fig. 3A. There is apparently no change in the surface ultrastructure of the oocyte in the vicinity of acrosome-reacted spermatozoa until about 1 min post-insemination.

Between 1 and 2 min post-insemination various stages of sperm incorporation were found. The spermatozoon enters perpendicularly and the oocyte cytoplasm becomes raised up around the sperm nucleus in a cone (Fig. 3B). The plasma membrane of the cone lacks microvilli and the cytoplasm lacks organelles, as shown also by Runnström (1963). The cortical granules (arrows) present in the deeper layer of cytoplasm remain intact. During the following 5 min the sperm head rotates 90° to the plane of entry and the cone continues increasing in size to form a typical bleb. The cytoplasm of the bleb is free of large organelles and again there is no obvious change in the structure of underlying granules (Fig. 3C). Spermatozoa also penetrate small oocytes; however, in these cases there is usually only one entry and the resulting bleb is extremely elongated (Fig. 4A–C). There is no apparent decrease in size of the penetrated oocyte and the bleb may attain a volume of 4% of that of the oocyte. As seen in the electron microscope young oocytes have a large band of peripheral cytoplasm (10 μm) that is virtually free of large organelles, and, in agreement with a previous report (Anderson, 1968), can be seen to contain cortical granules (arrows, Fig. 4D). It appears that the length of the bleb is determined, in part, by the length and number of microvilli on the oocyte surface.

**Electrical events**

As shown previously (De Felice & Dale, 1979) each sperm entry in *P. lividus* is marked by a step depolarization several mV in amplitude (Fig. 5B). However, in *S. granularis*, owing to the I–V characteristics of the membrane, the first sperm usually induces a large regenerative spike of about 50 mV amplitude (Fig. 5A). At high sperm densities (>10^7/ml) up to 10–15 sperm enter and the step depolarizations are practically superimposed.

To observe the behaviour of individual spermatozoa densities of 10^3 to 10^5/ml were used. Not all sperm that attached to oocytes gave rise to a depolarization. Those that did not induce a step depolarization continued gyrating around their point of attachment; a bleb did not form and the spermatozoon did not enter the oocyte. Penetrating spermatozoa gave rise to a step depolarization within 2–3 s of attachment and, in *S. granularis*, gyrated for approximately 50 s at 20°C. They then stiffen, perpendicular to the oocyte surface, and the bleb forms. With two intracellular micro-electrodes we have studied the conductance changes induced by spermatozoa (Fig. 5A, C). Each depolarization is accompanied by a decrease in input resistance of the oocyte of 20–40% (n = 10, e.g. see Fig. 5A). In cases of several successful interactions the input resistance decays progressively relative to the number of sperm entries. In the experiment shown in Fig. 5C there were five sperm entries and the input resistance decreased from 57 MΩ to about 10 MΩ, as if each sperm induced a decay in input resistance of about 30%. In the case of successful sperm–oocyte interactions the membrane
Fig. 4. A–C. Bleb growth in a young oocyte of *S. granularis* at 20°C. Bar 10 μm. Note that the bleb reaches a length of 30 μm. D. An electron micrograph to show the surface structure of a young oocyte. The microvilli are extremely long and frequent and the periphery of the cell is virtually free from large organelles. There is an extensive endoplasmic reticulum and the cortical granules (arrows) are present deep in the cytoplasm. × 12,600.
Fig. 5. Electrical responses to insemination in sea-urchin oocytes. A-C. Responses that resulted in sperm incorporation. D-F. Abortive 'switch-off' responses induced by non-penetrating sperm. The horizontal scale bar in A represents: 5 s in A, B and C; 10 s in D, E; 20 s in F. The vertical scale bar represents: 8 mV in A-D, F; 4 mV in E.

A. Voltage response in *S. granularis*; four blebs were later observed. Resting potential \((R_p) = -70 \text{ mV}\). B. Resistance drop at single step in *P. lividus* (5 MΩ to 3 MΩ). \(R_p = -40 \text{ mV}\); injected current \((I)\) (lower trace) = \(1 \times 10^{-9} \text{ A}\). C. Resistance decay in oocyte of *S. granularis* induced by five successful spermatozoa (57 MΩ to 10 MΩ). \(R_p = -40 \text{ mV}\); \(I = 1.4 \times 10^{-10} \text{ A}\). D. Voltage responses induced by unsuccessful spermatozoa (32 MΩ to 7 MΩ). \(R_p = -72 \text{ mV}\). E. Switch-off conductance events. The values refer to oocyte input resistance in MΩ. \(R_p = -50 \text{ mV}\); \(I = 1.4 \times 10^{-10} \text{ A}\). F. Multiple switch-off conductance changes in *S. granularis* (32 MΩ to 7 MΩ). \(R_p = -70 \text{ mV}\); \(I = 1.4 \times 10^{-10} \text{ A}\).
resistance and potential gradually return to about their pre-insemination values over several minutes in *P. lividus* and up to 20 min in *S. granularis*.

On many occasions we observed a third type of interaction: spermatozoa that induced apparently normal depolarizations; however, the potential abruptly returned to the resting level within 20–60 s (Fig. 5D). In these cases the spermatozoa continued gyrating around their point of attachment and blebs did not develop. Some of these unsuccessful spermatozoa broke away from the oocyte, others eventually fell motionless to the oocyte surface. The increase in conductance induced by these spermatozoa is comparable to that induced by successful spermatozoa. Fig. 5E shows single interactions in which the first spermatozoon causes the input resistance of the oocyte to decrease from 25 MΩ to 18 MΩ. After 40 s the resistance abruptly increased back to 25 MΩ. Later a second spermatozoon induces a decrease to 18 MΩ, while a third drops the resistance further to 14 MΩ. This latter resistance change is abruptly reversed after about 50 s. In the example shown in Fig. 5F, five spermatozoa attached almost simultaneously to the oocyte inducing a decay in resistance from 32 MΩ to about 7 MΩ, which reversed after 50 s.

It has been shown that in the presence of Quercetin, an ATPase inhibitor, spermatozoa attach to sea-urchin eggs and undergo the acrosome reaction; however, sperm incorporation is inhibited (Eckberg & Perotti, 1983). A similar effect was observed by inseminating eggs in Mg²⁺-free sea water (Sano et al. 1980). Quercetin at 100 μM blocks sperm incorporation in oocytes; furthermore, despite apparently normal attachments, the spermatozoa do not induce any electrical changes across the oocyte plasma membrane. Removal of Mg²⁺ has a similar effect. An example is shown in Fig. 6A. Partial replenishment of Mg²⁺ will lead to the regeneration of several abortive steps. Blebs, however, do not develop indicating that the spermatozoa are not incorporated. The effects of Mg²⁺ depletion and Quercetin are reversible.

When spermatozoa are diluted into natural sea water (NSW) they gradually and progressively lose their fertilizing ability. *S. granularis* spermatozoa are not viable after 10 min dilution at 20 °C, even though they are motile and are capable of attaching to oocytes and eggs. If diluted for a shorter period (5–9 min) the spermatozoa give rise to abortive steps (e.g. see Fig. 6B).

In order to investigate the role of the acrosomal contents of the spermatozoa in the events of sperm–oocyte interaction, three standard methods of inducing the acrosome reaction were used: (1) Diluting spermatozoa in NSW at pH 9·6; (2) diluting spermatozoa into NSW containing jelly solution prepared from eggs; and (3) pretreating diluted spermatozoa in 20 μM of the calcium ionophore A23187. All three treatments caused sperm agglutination (Epel, 1978). Such spermatozoa, if used within 1 min of treatment, were viable and gave rise to normal steps and blebs. Since the divalent cation ionophore A23187 induces fertilization membrane formation in sea-urchin eggs (Steinhardt & Epel, 1974) and a depolarization of the egg plasma membrane (Dale & de Santis, 1981), its effect on oocytes was studied. A23187 at 20 μM did not appear to have any effects on the gross morphology of oocytes as observed in the phase-contrast microscope and in four out of eight oocytes had no effect on the resting potential. In four other oocytes the ionophore induced a gradual depolarization of
Fig. 6. A. The role of Mg$^{2+}$ in sperm–oocyte interaction in the sea-urchin. An oocyte in low-Mg$^{2+}$ (5 µM) sea water was impaled and inseminated with spermatozoa diluted in Mg FSW (first arrow). Although spermatozoa attached to the oocyte there were no electrical or morphological changes. At the second arrow a drop of sea water was added to partly replenish the Mg$^{2+}$ and several of the spermatozoa generated abortive steps, which did not lead to bleb formation. At the third arrow fresh sperm diluted in natural sea water were added and these gave rise to normal electrical events and blebs; $R_p = -60$ mV. B. The effect of ageing spermatozoa on the electrical events of insemination. Spermatozoa at 10$^7$/ml diluted in NSW for 5 min were added to an impaled oocyte (first arrow). Three abortive steps were recorded and blebs were not observed. Later, spermatozoa from the same batch at 10$^7$/ml were added to the oocyte (second arrow) 2 min after dilution. Normal electrical and morphological events were observed; $R_p = -70$ mV. C. Annulment of sperm-induced depolarizations by the addition of 2% glutaraldehyde to the impaled oocyte; $R_p = -80$ mV. Note that the potential first returns to its resting level and then slowly depolarizes concomitantly with cell death. The vertical scale bar in A represents 8 mV for A, B, C; while the horizontal scale bar represents 20 s for: A, C; 10 s for B.
1 mV/min, shifting the resting potential from $-80$ to $-50$ mV. Finally, we observed that oocytes exposed to $20 \mu M$-A23187 for several minutes and then inseminated in the presence of the drug gave rise to normal step events and blebs.

At some point in sperm-oocyte interaction fusion of the plasma membranes of the two gametes must occur. Although the spermatozoon is 1/500 000 of the volume of the oocyte, this event could contribute to the electrical changes observed across the oocyte plasma membrane following insemination. That is, at the moment of fusion the conductance of the spermatozoon plasma membrane would effectively be in parallel to that of the oocyte. In order to investigate this possibility, ideally one would like to remove the spermatozoon at various intervals following its attachment to the oocyte surface. In this report an alternative method was used. Oocytes were impaled and inseminated and then at various intervals following the generation of the sperm-induced depolarizations a drop of 2% glutaraldehyde or, alternatively, of the detergent sodium lauryl sulphate (0.1%) was added to the oocyte. Owing to the vast difference in size of the gametes, the attached spermatozoa are essentially fixed or destroyed almost immediately, whereas the oocyte remains viable for many seconds longer (e.g. see Fig. 6c). The oocyte inseminated at $10^7$/ml gave rise to eight practically super-imposed step depolarizations. At the arrow a drop of 2% glutaraldehyde was added and 3 s later the potential rapidly hyperpolarized, returning to its original pre-insemination resting value of $-80$ mV. At 25 s later the membrane slowly depolarized at 20 mV/min, eventually reaching zero mV. In three oocytes two intracellular electrodes were used to measure membrane resistance ($R_m$). As shown previously (Fig. 5) the $R_m$ decreases during the sperm-induced depolarization. On addition of glutaraldehyde, and simultaneously with the abrupt hyperpolarization, the $R_m$ increased, attaining its original pre-insemination value. During the later slow depolarization, which probably indicates cell death, the $R_m$ progressively increased. Glutaraldehyde also annuls the sperm-induced depolarizations when added 4 min after their initiation, during the growth period of the blebs. Finally, sodium lauryl sulphate has the same effect as glutaraldehyde; however, if it is carefully diluted away following the experiment the oocyte retains its original electrical characteristics and remains viable.

**DISCUSSION**

The first change in the sea-urchin oocyte during interaction with a viable spermatozoon is a small step depolarization accompanied by a decrease in resistance. There is no further change until 50 s later (at 20°C), when the spermatozoon stops gyrating, the oocyte surface swells up at the point of sperm attachment and the sperm nucleus disappears within this cytoplasmic bleb. Each successful spermatozoon induces a 20–40% decay in input resistance across the oocyte plasma membrane and consequently in cases of multiple sperm entries the resistance decreases progressively. Not all of the attached spermatozoa, however, give rise to this electrical event; those that do not are unsuccessful and do not enter the oocyte. The reason for this is not clear. It could be due to differences in maturity of the spermatozoa or, alternatively, there may be a limited number of sites on the oocyte surface available for sperm penetration.
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We raise the possibility that this primary electrical event in the sea-urchin oocyte at insemination is the result of sperm-oocyte fusion; the ion channels that give rise to the conductance change measured across the sperm-oocyte complex being located in the sperm plasma membrane. There are several lines of evidence to support this argument.

First, the observation that the sperm-induced electrical events may be annulled by killing the spermatozoa with fixative or detergent. Second, the fact that in the presence of Quercetin the spermatozoa attach to the oocytes in an apparently normal fashion and undergo the acrosome reaction, although they do not fuse or generate any electrical change. Finally, under certain conditions the sperm-induced conductance change may spontaneously reverse; i.e. it switches off, and as a consequence sperm entry is prevented. Using conventional voltage clamp techniques, Lynn & Chambers (1984) have shown that sea-urchin eggs clamped at \(-30\) to \(-80\) mV generate small transient currents upon insemination, which switch off after several seconds. These current blips are initiated by non-penetrating spermatozoa. It is probable that the step conductance event in sea-urchin oocytes described in this report is analogous to the primary electrical event observed in mature eggs at fertilization (Dale et al. 1978).

An important question is whether the conductance change observed across the oocyte-sperm complex triggers later events leading to sperm entry and, in the case of eggs, activation. In the present study we observed no change in the oocyte surface until about 50 s post-insemination, when the spermatozoon stiffens and the oocyte cytoplasm swells to form a bleb. Unsuccessful spermatozoa may give rise to similar conductance changes; however, the latter events do not occur and the conductance change reverses. This suggests that the primary conductance change does not trigger later events but that a second determinative event occurs at 50 s. Comparable changes occur during the fertilization of eggs; however, the time-scale is somewhat different. In eggs the spermatozoon immobilizes about 13 s after the generation of the first electrical event (Dale & de Santis, 1981), and shortly afterwards is engulfed by the fertilization cone and the fertilization membrane elevates. The delay in bleb formation in the oocyte is probably due to the fact that the spermatozoon is triggered into the acrosome reaction rather later.

What is this second event that leads to the formation of the cytoplasmic protrusion and sperm incorporation? It has been shown elsewhere that the spermatozoon contains a soluble factor that will trigger cortical granule exocytosis when pressure-injected into sea-urchin eggs (Dale, De Felice & Ehrenstein, 1985). This factor probably induces the release of \(\text{Ca}^{2+}\) from intracellular stores in the egg. We have no evidence that the immature oocyte has stores of intracellular \(\text{Ca}^{2+}\) or if these are mobilized during insemination; however, it is feasible that \(\text{Ca}^{2+}\) is a second messenger in the process leading to bleb formation. First, in order to grow pressure must be generated within the cytoplasmic protrusion. It has been suggested that ground cytoplasm flows from the cell interior to the area of sperm entry (Runnström, 1963; Lönn, 1965). Alternatively, we raise the possibility that the total volume of the cell may increase following sperm penetration and this implies an influx of water from the
environment. Both of these processes may be regulated by intracellular Ca\(^{2+}\) (Heilbrunn, 1956). We suggest that this second event results from the transfer of a soluble component from the spermatozoon into the oocyte cytoplasm. This component inducing a localized release of stored Ca\(^{2+}\). It is tempting to speculate that the soluble sperm component is stored in the periacrosomal vesicles, first described by Franklin (1965).

REFERENCES


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(Received 24 July 1984 – Accepted 26 September 1984)