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SPERM RECEPTIVITY IN SEA URCHIN OOCYTES AND EGGS

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SUMMARY

An electrophysiological method for studying the receptivity to spermatozoa of sea urchin eggs and oocytes is described. Pairs of adjacent oocytes and eggs, with intact jelly layers, were impaled and simultaneously exposed to a known concentration of spermatozoa. Two parameters were studied. The time from insemination to the first successful collision (as indicated by a step depolarization across the egg plasma membrane) and the total number of successful collisions. Sperm densities of $10^7\,ml^{-1}$ were used, which ensured almost immediate interaction between several thousand spermatozoa and each female gamete. In all cases, under control conditions, the oocyte was more receptive to spermatozoa than was the egg, giving rise to the first electrical event at about 5 s post-insemination, compared to about 13 s for the egg. In addition, whereas 10–15 spermatozoa usually entered the oocyte, only one entered the egg. The low receptivity of eggs appears to be independent of resting potential. Cytoplasmically immature eggs or mature eggs briefly exposed to nicotine, strychnine, choline or Tris tend to be polyspermic and have comparable receptivity to oocytes. The data suggest that there are a limited number of viable interaction sites on the oocyte surface and that during cytoplasmic maturation these sites are rendered less receptive. In the mature egg there may be one preferential sperm entry site. This hypothesis is consistent with the experimental data and would explain why sea urchin eggs are usually monospermic at high sperm densities.

INTRODUCTION

Rothschild & Swann (1949, 1950, 1951, 1952; Rothschild, 1953) were the first authors to quantify the receptivity of sea urchin eggs to spermatozoa. They devised a method for calculating the rate of appearance of fertilized eggs in a population of eggs exposed to a known concentration of spermatozoa. This rate is an indirect estimate of receptivity. Several authors have since used this method, with various modifications, to study different aspects of sperm-egg interaction in sea urchins (Hagström, 1956; Hagström & Runnström, 1959; Presley & Baker, 1970; Byrd & Collins, 1975).

Key words: Echinoderm, eggs, polyspermy.

In the present report, the sperm receptivity of individual sea urchin eggs and oocytes is examined by a direct electrophysiological method. I used this new direct technique of monitoring receptivity to investigate the effects of the polyspermy-inducing agents, strychnine and nicotine (Hertwig & Hertwig, 1887), and to determine whether choline or Tris had any effects on this parameter. Experiments, carried out at ambient temperature using eggs and oocytes with intact jelly layers, show that the sperm receptivity of sea urchin eggs is independent of the egg's resting potential and is probably regulated by properties of the egg surface.

MATERIALS AND METHODS

Experiments were carried out on gametes of the sea urchins *Paracentrotus lividus, Psammechinus microtuberculatus* and *Sphaerechinus granularis* collected from the Bay of Naples. The gametes were obtained by dissection and stored at room temperature (18–22 °C). Spermatozoa were kept in the 'dry' state until immediately before each experiment when they were diluted using natural sea water (NSW). Sperm density was calculated using a haemocytometer. Batches of eggs containing oocytes were selected: in the case of *Sphaerechinus*, the gonads were teased apart using forceps to release the immature gametes. In the majority of cases eggs and oocytes were used with their jelly layers intact. To remove the jelly, eggs were suspended in NSW at pH 5·5 for 2 min and then washed twice in NSW.

Electrical recordings were made on eggs and oocytes on glass slides, to which the gametes did not adhere. In each experiment an egg and an adjacent oocyte were impaled. The jelly layers of the cells usually spaced them apart by about 100 μm. Insemination was carried out as follows. A drop of freshly diluted spermatozoa at the desired density was released directly over the impaled cells which were covered by a minimal amount of NSW. In this way the impaled cells were simultaneously exposed to a known concentration of spermatozoa. Microelectrodes of 40–80 MΩ resistance filled with 1·8 mol l^{-1} potassium citrate, were used for the intracellular recordings, which were stored on FM tape for subsequent analysis.

All experiments were carried out in NSW at 18–22 °C. Nicotine (BDH, England) and strychnine (BDH, England) were made up directly in NSW at the desired concentration. Choline chloride (Sigma, St Louis) was used to replace Na^+ in artificial sea water solutions. The ASW had the following composition (mmol l^{-1}): NaCl, 575; $MgSO_4$, 55; $CaCl_2$, 11; KCl, 10; $NaHCO_3$, 2.

RESULTS

In each experiment, an egg and an adjacent oocyte were impaled and their resting potentials recorded. The cells were then simultaneously exposed to a sperm suspension and their responses to insemination monitored. Over 90

experiments on eggs and oocytes from 21 different batches were carried out. Medium to high sperm densities were used (10^6 ml^{-1} to 2×10^7 ml^{-1}) to ensure a rapid interaction between sperm and eggs.

A typical experiment at medium density is shown in Fig. 1A. In the oocyte the majority of attached spermatozoa were unsuccessful, and did not induce any electrical events or enter the oocyte (B. Dale & L. Santella, in preparation; see Fig. 6). Each successful spermatozoon collision is marked by a step depolarization, which gave rise to a spike in *S. granularis*. The first spike occurred at about 15 s after insemination followed by four more at intervals of some 30 s. In

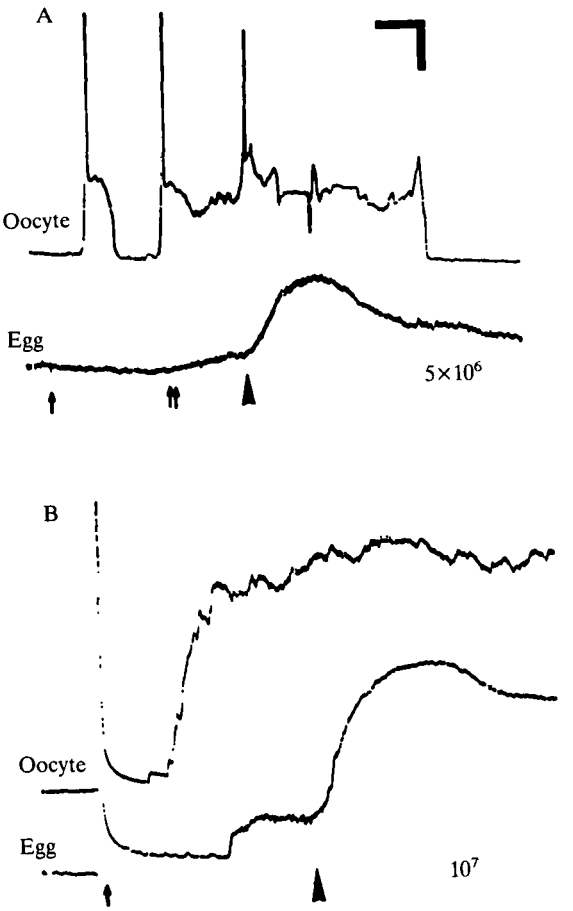

Fig. 1. Fertilization potentials recorded simultaneously from sea urchin eggs and oocytes. (A) *Sphaerechinus granularis*, resting potential of oocyte (RP) −80 mV, RP of egg −20 mV. (B) *Psammechinus lividus*, RP of oocyte −60 mV; RP of egg −32 mV. Note that the oocyte responds earlier than the egg. In (A) the small step depolarization in the egg is indicated by the double arrow. The large arrowhead marks the initiation of the cortical reaction in the egg. Horizontal bar: 20 s for (A), 5 s for (B). Vertical bar: 20 mV for oocyte (A), 8 mV for egg (A), oocyte (B) and egg (B). Temperature, 20 °C. In these, and all subsequent recordings, time of insemination is indicated by a single small arrow and sperm density (number ml^{-1}) is indicated in numbers.

contrast, despite the fact that many spermatozoa attached to the surface of the egg, only one successful collision occurred at 50 s post-insemination (double arrow). If, as in the case of the oocyte, successful collisions had occurred at a linear rate, then a second collision would be precluded by the cortical reaction which is initiated about 25 s after the first collision (arrowhead).

A second experiment using gametes of the sea urchin *P. lividus* at a higher sperm density is shown in Fig. 1B. Many spermatozoa attached to both cells almost immediately. In the oocyte the successful collision rate was high, making it difficult to count individual steps. Nevertheless, the first successful encounter occurred at 5 s post-insemination and was followed by a delay of almost 2 s: several additional, almost super-imposed, steps then occurred. In the case of the egg, the only successful collision occurred at 13 s post-insemination. This egg was monospermic.

The time lag from insemination to the first electrical event is an indication of receptivity to spermatozoa. In all experiments on untreated material we found that the oocyte is more receptive to spermatozoa than is the egg. There was much variation between batches of eggs. Moreover, batches from animals collected in the spring seemed to be more receptive than batches collected in the autumn. This parameter of receptivity of course depends also on the sperm batch, the sperm density, the percentage of physiologically mature spermatozoa, and factors in the seminal fluid. For these reasons of variability, we did not statistically analyse our data. Instead, we present data from five batches of eggs (Table 1). At sperm densities of about $10^7\,ml^{-1}$, the time lag ranged from 2–5 s for oocytes and from 7–25 s for eggs. In addition, whereas 10–15 spermatozoa usually entered the impaled oocyte, only one entered the impaled egg.

It is known that the jelly layer slows the fertilization rate at medium sperm densities (Hagström, 1956). That is, carefully prepared, jelly-free eggs are more receptive to spermatozoa than are intact eggs. At high densities, the jelly layer seems to have little effect on the fertilization rate (L. J. De Felice & B. Dale, in preparation) and in fact in the present study the time lag between insemination and the first electrical step was comparable in jelly-free and jelly-intact eggs (nine experiments, see Table 1).

Cytoplasmic maturity and egg receptivity

Animals collected before the peak of the breeding season often contained large numbers of oocytes (>20 %). Eggs from such animals are considered to be cytoplasmically immature and are known to be prone to polyspermy (Runnström, 1949). Such 'immature' eggs had low resting potentials in the range −8 to −30 mV and were found to be as highly receptive as the oocytes (Fig. 2A).

The difference in receptivity between cytoplasmically mature eggs and oocytes persists for at least 25 h, which is the longest period eggs may be maintained *in vitro* without excessive deterioration. Even so, such eggs had spontaneously lost a fraction of their cortical granules and, in fact, upon insemination the second

Table 1. *The time lag between insemination and the first electrical response in sea urchin eggs and oocytes*

Species	Resting potential (mV) Egg	Oocyte	Time to step (s) Egg	Oocyte	Age (h)	P	J	FM	Sperm density (ml^{-1})
S	−20	−22	10	5	1	m	−	+	2×10^7
S	−32	−80	25	5	2	p	−	−	2×10^7
S	−55	−90	21	5	6	p	−	−	2×10^7
S	−20	−80	50	15	7	m	−	+	5×10^6
S	−30	−70	40	25	8	m	−	+	5×10^6
S	−24	−48	7	5	2	p	+	+	10^7
S	−32	−80	17	12	3	m	+	+	10^6
S	−32	−60	8	3	3	p	+	+	5×10^6
Pa	−27	−90	25	5	1	m	+	+	10^7
Pa	−16	−80	22	5	3	m	+	+	10^7
Pa	−32	−60	23	5	6	m	+	+	10^7
Pa	−24	−80	10	3	6	p	+	−	10^7
Pa	−24	−80	13	4	7	p	+	−	10^7
Pa	−60	−80	30	3	25	m	−	−	2×10^7
Pa	−32	−80	20	3	25	m	−	−	2×10^7
Ps	− 8	−20	7	2	1	m	+	+	10^7
Ps	−10	−80	9	2	1	m	+	+	10^7
Ps	− 8	−80	10	4	2	m	+	+	10^7
Ps	−12	−80	7	5	2	m	+	+	10^7
Ps	−16	−70	10	3	1	m	+	+	2×10^7
Ps	−18	−90	12	3	4	m	+	+	2×10^7
Ps	−22	−90	10	3	4	m	+	+	2×10^7

Species: *S*, *Sphaerechinus*; *Pa*, *Paracentrotus*; *Ps*, *Psammechinus*.
Age = time gametes are *in vitro*.
P, polyspermic (p) or monospermic (m) condition of egg as determined at first cleavage: J, presence of jelly layer; FM, elevation of a normal fertilization membrane.
Temperature = 18–22 °C.

electrical depolarization was consistently reduced in amplitude (Fig. 2B). Eggs that were aged for several hours tended to become slightly more receptive, as indicated by a decrease in the time interval from insemination to the first electrical event (Table 1).

The effects of nicotine and strychnine on egg receptivity

Pre-treatment of eggs with the alkaloids, followed by several washes in NSW and insemination in NSW

In three control experiments the oocytes responded to insemination within 3–4 s and the eggs within 11–25 s. Pulse treatment of eggs with the alkaloids increased their receptivity, making them comparable to oocytes (Fig. 3A; six experiments). In these eggs the resting potentials were unaltered (−8 to −30 mV) and they raised normal fertilization membranes. They were always polyspermic. Two minutes pre-treatment was sufficient to induce this change in receptivity.

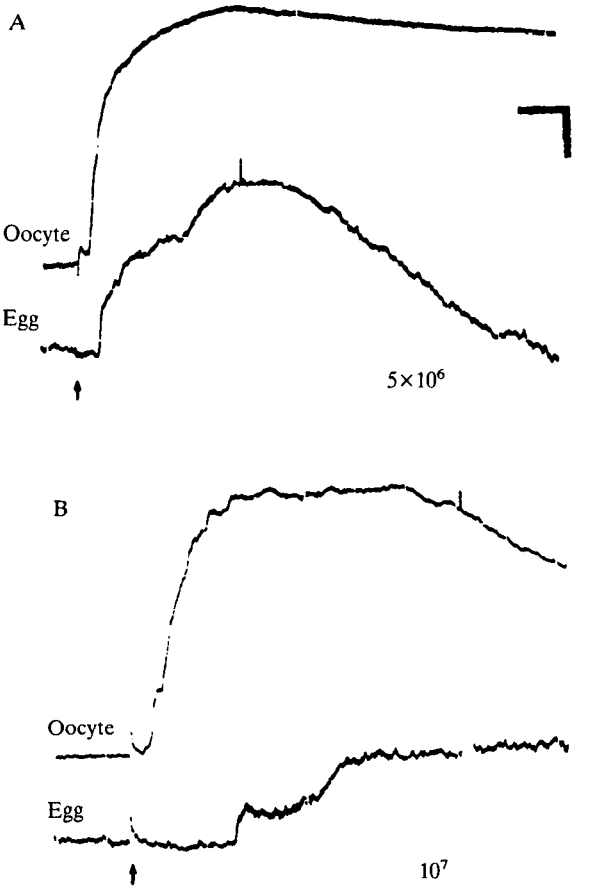

Fig. 2. (A) A typical experiment from an immature batch of eggs. Note the egg (RP, −32 mV) is as receptive as the oocyte (RP, −60 mV) and was polyspermic. *Sphaerechinus granularis*. (B) The effect of ageing on egg receptivity (25 h *in vitro*). Note the oocyte (RP, −80 mV) gives rise to an apparently normal response, while the egg (RP, −32 mV), although less receptive, gives rise to an abnormal response. *Psammechinus lividus*. Horizontal bar: 10 s for (A); 5 s for (B). Vertical bar: 8 mV for all traces.

Pre-treatment of eggs with the alkaloids and fertilization in the presence of the alkaloid

Experiments on nicotine have been described elsewhere (see Dale, de Santis & Hagström, 1982). Essentially, extensive exposure to this drug resulted in polyspermy due to an increased receptivity but also to impairment of the cortical reaction. Strychnine affected fertilization more severely. In three experiments carried out in 1 mmol l^{-1} strychnine, we could not detect any electrical events in the egg (Fig. 3B) and fertilization membrane elevation was suppressed: nevertheless, sperm did enter the eggs as shown by large fertilization cones.

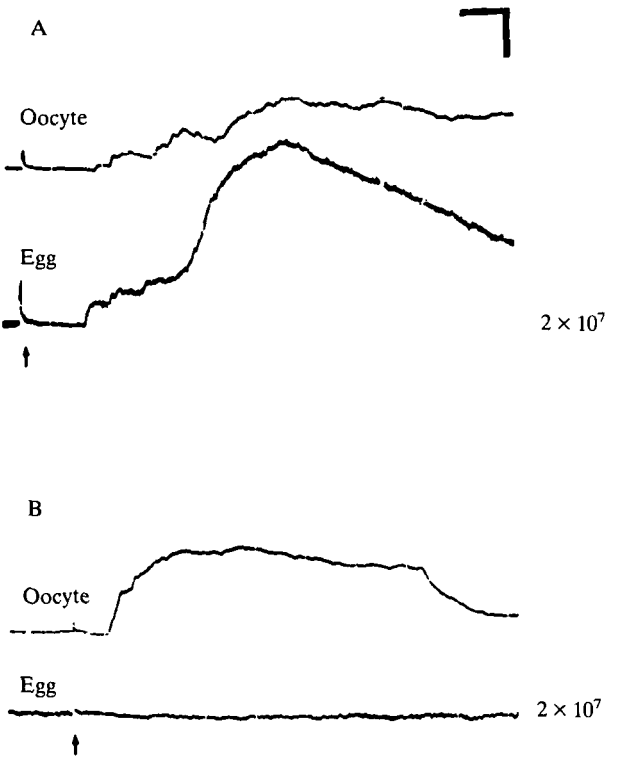

Fig. 3. The effect of strychnine on sperm receptivity in sea urchin eggs and oocytes. (A) Pre-treatment in 1 mmol l^{-1} strychnine for 6 min. Note the egg and oocyte are equally receptive. (B) Fertilization in the presence of 1 mmol l^{-1} strychnine. Although there are no electrical events in the egg and no cortical reaction, several spermatozoa enter as shown by multiple penetration cones. Vertical bar: 20 mV for the oocytes, 8 mV for the eggs. Horizontal bar: 5 s. *Psammechinus lividus*.

The effects of choline and Tris on sperm receptivity

Pre-treatment of eggs in choline sea water (Na^+ content about 50 mmol l^{-1}), washing and then fertilization in NSW

Results from one batch are shown in Fig. 4. The control egg was, as always, less receptive than the oocyte (Fig. 4A). A 5-min exposure of the eggs to choline sea water followed by two washes and fertilization in NSW resulted in an increased receptivity, without any alteration in the resting potential (Fig. 4B). Exposure for longer periods (up to 30 min) resulted in an increase in the resting potential of the egg to about −80 mV. These high resting potential eggs, when inseminated in NSW, gave rise to a rapid overshooting depolarization which reached +20 mV (Fig. 4C). Such eggs were highly receptive, responding within 2 s of insemination, and raised perfect fertilization membranes. In Fig. 4C, note that the oocyte received fewer successful collisions, somewhat spread out over time. This experiment was repeated three times and in each case the same type of fertilization response was obtained.

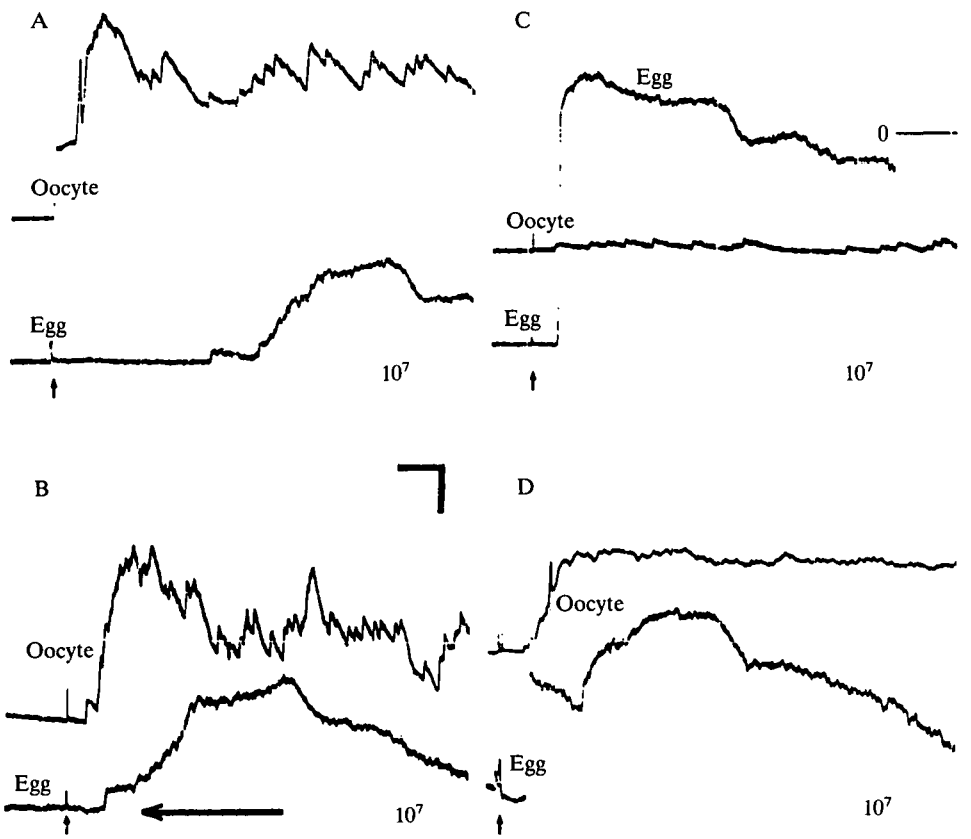

Fig. 4. The effects of choline and Tris on egg receptivity. (A) Control oocyte, RP −80 mV; egg, RP −14 mV. (B) Eggs pre-treated for 5 min in choline sea water (Na^+ content approx. 25 mmol l^{-1}), washed and inseminated in NSW. Note the increased receptivity of the egg; oocyte, RP −80 mV; egg, RP −16 mV. (C) Eggs pre-treated for 30 min in choline sea water, washed and inseminated in NSW; oocyte, RP −80 mV; egg, RP −80 mV. (D) Eggs pre-treated for 30 min in sea water containing 10 mmol l^{-1} Tris washed and then inseminated in NSW; oocyte, RP −80 mV; egg, RP −32 mV. Note the increased receptivity of the egg. *Psammechinus lividus*. Horizontal bar: 5 s. Vertical bar: 8 mV for (A) and (B); 16 mV for (C); 20 mV for oocyte in (D) and 8 mV for egg in (D).

Pre-treatment of eggs in choline sea water and fertilization in choline sea water

When fertilization was carried out in the presence of choline sea water (three experiments), the eggs were as receptive as for pulse treatment. However, the fertilization potentials were reduced in amplitude, as reported elsewhere (Jaffe, 1980).

Tris has similar effects on fertilization to choline, nicotine and strychnine. Brief exposures of unfertilized eggs to 10 mmol l^{-1} Tris resulted in an immediate increase in egg receptivity (three experiments) without change in resting potential. A more prolonged exposure (>10 min) resulted in an increased resting potential and an increase in receptivity. Pre-treatment of eggs for 10 min in 10 mmol l^{-1} Tris in sea water followed by washing and fertilization in NSW also

resulted in an increased receptivity (Fig. 4D; three experiments). Eggs pretreated or fertilized in 10 mmol l^{-1} Tris raised normal fertilization membranes and, when they were not polyspermic, cleaved normally.

The effect of sperm density on egg receptivity

In 1981 Dale & de Santis demonstrated that the first electrical event at fertilization occurs 2–4 s after the attachment of the fertilizing spermatozoa to the egg surface. This experiment was repeated by Schatten & Hülser (1983) who obtained the same result. In both these reports very low densities of spermatozoa were used in order to identify the fertilizing spermatozoon.

In the present experiments high densities of spermatozoa were used (10^7 ml^{-1}). Under such conditions many thousands of spermatozoa penetrated the jelly and attached to the egg surface within a few seconds. In fact, the adjacent oocyte provided evidence of rapid exposure as it responded electrically to spermatozoa within 5 s. Why then, in contrast to the result of Dale & de Santis (1981), did the impaled egg not respond to the fertilizing spermatozoon for some 10–20 s? One possibility is that a factor in the sperm medium slows down the processes leading to the generation of the electrical event. To test this idea several experiments at extremely high densities (5×10^8 ml^{-1}) were carried out. An example is shown in Fig. 5. It can be seen that at these very high densities the step event is delayed compared to medium densities. Fig. 5C is a photograph of the experiment shown in Fig. 5B, demonstrating that the impaled egg behaved as the surrounding unimpaled eggs. A component of the sperm medium could be a regulatory factor involved in sperm-egg interaction, or alternatively, if we consider that these high densities are non-physiological, then perhaps the slowing effect is an artifact caused by the same factors that serve to inhibit sperm motility and activity during storage in the testis.

DISCUSSION

The experiments in this paper show, by a direct method, that sea urchin eggs are less receptive than oocytes to spermatozoa and that 10–15 spermatozoa enter oocytes, whereas usually only one spermatozoon enters the egg. Eggs from immature animals, that have probably not completed cytoplasmic maturation, or mature eggs treated briefly in nicotine, strychnine, choline or Tris are as receptive as oocytes and tend to be polyspermic when inseminated at sperm densities that cause little polyspermy in control eggs. This induced change in receptivity occurs independently of any changes in resting potential of the egg. The data suggest that sperm receptivity in sea urchin eggs and oocytes is not correlated with the resting potential.

It has been known for some time that alkaloids increase the receptivity of sea urchin eggs as shown by an increased fertilization rate (Rothschild, 1953; Hagström, 1956; Hasträm & Allen, 1956; Dale *et al.* 1982). This effect has been quantified here using an electrophysiological parameter and, in addition, it has

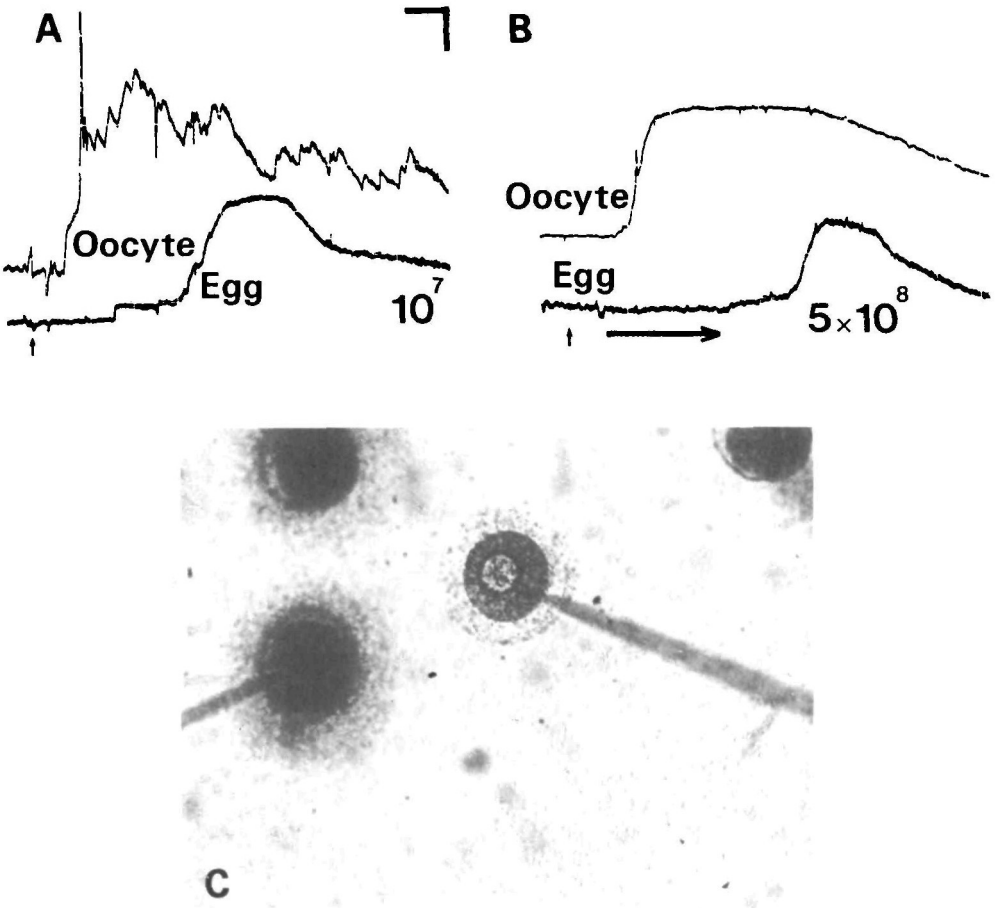

Fig. 5. The effect of sperm density on receptivity. (A) Control at $10^7\,ml^{-1}$. (B) Same batch inseminated at 50 times this concentration. Note the decrease in egg receptivity. (C) Photograph of eggs and oocytes in (B) at 41 s post-insemination. Note that the impaled egg had raised a fertilization membrane at about the same time as the surrounding eggs. The impaled egg cleaved normally. Magnification, (× 100). Horizontal bar: 5 s. Vertical bar: 8 mV for (A) and egg in (B) and 20 mV for the oocyte in (B).

been shown that both nicotine and strychnine render eggs as receptive as oocytes. Tris and choline, which are often used in experiments on sea urchin gametes (Chambers & de Armendi, 1979; Jaffe, 1980; Schuel & Schuel, 1981), also increase the receptivity of sea urchin eggs. At present there is no indication as to why these four weak amine bases alter the sperm receptivity of sea urchin eggs. One possibility is that they alter the intracellular pH of the egg and this renders the egg more receptive. An alternative explanation is that sperm-egg interaction is regulated by surface charge and these positively-charged molecules in some way modify the existing membrane surface charge by screening or binding.

Sea urchin eggs in a healthy mature condition are usually monospermic when fertilized at high sperm densities. It is the current consensus that eggs are

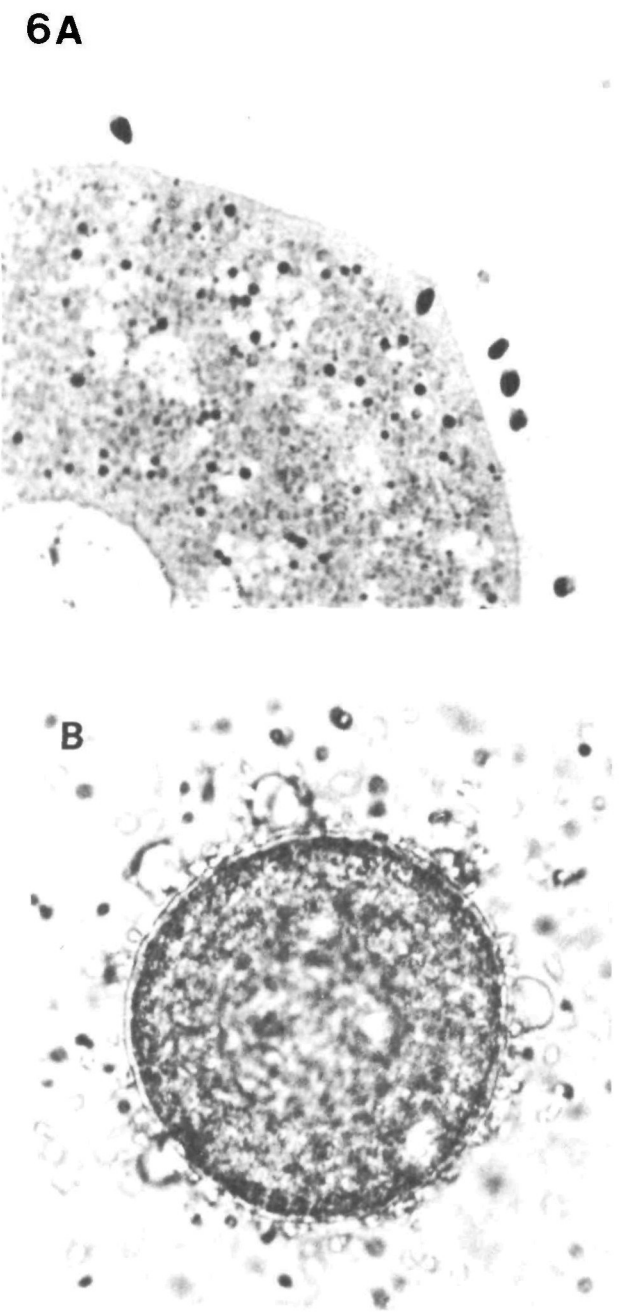

Fig. 6. (A) A semi-thin section of an oocyte inseminated at $10^7\,ml^{-1}$. One spermatozoon has entered and induced a fertilization cone, others remain attached to the surface (× 11 000). (B) A photograph of a live oocyte 3 min after insemination at a sperm concentration of $10^7\,ml^{-1}$. Although several thousand spermatozoa attach, note that there are only four fertilization cones. Magnification, (× 700).

endowed with mechanisms to prevent the entry of supernumerary spermatozoa. While it is widely accepted that the cortical reaction will prevent the entry of spermatozoa, there is controversy over the existence of a second mechanism postulated by Rothschild & Swann (1952) and called the fast partial block. The present report does not directly address the question of the existence of a fast block (see reviews by Whitaker & Steinhardt, 1982; Nuccitelli & Grey, 1984; Dale & Monroy, 1981), however it does raise some interesting points which lead to an alternative hypothesis as to how sea urchin eggs prevent polyspermy.

Fig. 6 shows a photograph of an oocyte fertilized at a relatively high sperm density. It can be seen that of several thousand 'attachments' only a relatively few spermatozoa are successful and enter the oocyte (usually 10–15). Oocytes are not capable of a cortical reaction (the so-called slow block) and there is no evidence to suggest they have a fast partial block. Thus, in oocytes up to 99 % of sperm attachments are not effective.

This suggests that there are a limited number of sites on the oocyte surface through which spermatozoa can enter. It is possible that during cytoplasmic maturation there is a progressive decrease in the number of sperm penetration sites, the mature egg having one preferential entry site. However, mature eggs are sometimes polyspermic and can be made polyspermic by treatment with various chemical agents. This could mean that the sperm penetration sites removed during maturation are not lost completely but are merely made less receptive in some way, perhaps involving charged groups. The receptivity of each secondary site may also vary and depend on the viability of the particular spermatozoon interacting with it. It can be noted in some recordings (see Figs 1B, 2A,B, 4B) that in oocytes exposed to a dense suspension of spermatozoa, one spermatozoon often interacts with the oocyte a few seconds before the other successful spermatozoa. This may mean that there is already a preferential site for sperm entry on the oocyte surface.

The idea of a preferred pathway for sperm penetration in sea urchin eggs is not new; in fact Boveri (1901) suggested that the jelly canal at the animal pole was such a site. Runnström (1949, 1961) also held the view of a preferential entry site for sperm. This idea has been criticized by many authors who maintain that the site of sperm entry is random with respect to the animal pole (see Schroeder, 1980 for references). However, the fact that in a population of eggs the fertilizing spermatozoa appear to enter randomly as regards the A-V axis does not necessarily mean that spermatozoa may enter anywhere over the egg surface, but rather that there may be a preferential entry site located randomly on the egg surface.

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