MEMBRANE FUSION EVENTS IN FERTILIZATION

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INTRODUCTION

Fertilization entails the union of egg and spermatozoon, which, as first clearly shown by Szollosi & Ris (1961) in a mammal and Colwin & Colwin (1961) in an invertebrate organism, requires the fusion of the limiting membranes of the two cells so that they come to form a single cell. The new entity is bounded by a membrane which at least initially is a composite or mosaic of the two gamete membranes. Before gamete union can occur the spermatozoon must penetrate the investments enclosing the egg, and this requires the release of lytic enzymes carried in the acrosome. Release is effected by the acrosome reaction, which takes place by fusion between acrosomal and plasma membranes. The acrosome reaction must itself be preceded by changes in the surface properties of the spermatozoon, known collectively as capacitation, and this is likely to involve also changes in the sperm plasma membrane. After gamete union, the egg becomes protected by blocks to polyspermy, involving changes in the vitelline surface, and also in the zona pellucida in all mammals apparently except lagomorphs. Associated in time, and probably causally, with the blocks to polyspermy is the breakdown of the cortical granules, requiring fusion of their membranes with the egg plasma membrane. Accordingly, discussion on the membrane fusion events in fertilization requires consideration of four distinct processes: capacitation, the acrosome reaction, gamete fusion, and the breakdown of cortical granules. This review is concerned mainly with the observations that led to the present state of understanding of these processes as components of fertilization. In the other papers of the Symposium, attention is given to ultrastructural features of artificially produced acrosome reactions and to the molecular biology of membrane fusion events involving gametes and other cells.

CAPACITATION

The first evidence for capacitation was obtained independently by Austin (1951) and Chang (1951) in the rat and rabbit; the experimental procedure involved injection of sperm suspension into the ovarian capsule (rat) or into the oviduct (rabbit), and observations indicated that spermatozoa required to spend some time in the female tract before becoming capable of penetrating egg investments. The possibility still remained that the delay noted may have been to some extent artefactual, and so an experiment was set up involving only alteration in time relations: rats were permitted to mate 3 to 10 hr after ovulation, instead of at the usual time, about 8 to 10 hr before ovulation. Sperm penetration did not begin until 2 1/2 to 3 hr after mating, though spermatozoa reached the site of fertilization within 1/2 hr; penetration then proceeded rapidly
have receptors from Pinsker, a found data released labile rate, these vented in (1964) about 65°C by capacitation of atozoa be material Krebs-Ringer existence Atozoa were recovered decapacitated demonstrated. uterus, fluid (Austin 156) glycoprotein, Another establishment is oxygen & spermatozoa is, however, from 1967; those spermatozoa were recovered decapacitated demonstrated. The existence was postulated of an inhibitory substance (‘decapacitation factor’) in semen which became adherent to the surface of the spermatozoa and prevented them from taking part immediately in fertilization. Subsequent work by Chang and a number of other workers has generally confirmed and extended these observations, and the widely held view now is that an important step in capacitation lies in the removal of ‘stabilizing’ or ‘inhibitory’ extraneous material from the sperm surface. Identity of this material with decapacitation factor is, however, still speculative, and the reality of ‘recapacitation’ has yet to be established (the alternative possibility being an initial capacitation of spermatozoa that had previously resisted the change).

Removal of extraneous material may well affect permeability and metabolic rate, and it is therefore of interest that Iritani, Gomes & VanDemark (1969) found that oxygen uptake by spermatozoa was increased above that shown in Krebs-Ringer phosphate medium by the addition of oviducal fluid or especially of uterine fluid. The authors stressed the responsibility of a non-dialysable, heat-labile factor for the uterine effect. The possible role of glycolytic agents in capacitation is discussed later. The extraneous coat of the spermatozoon as released from the male tract is presumably deposited in the testis or epididymis; data on the importance of the epididymal contribution are reviewed by Johnson (1975).

Attempts were made to isolate and identify decapacitation factor. It was found to be removed from solution by ultracentrifugation, to be precipitable by treatment with 60% ethyl alcohol at −30°C, non-dialysable, and stable at 65°C (Bedford & Chang, 1962). Further studies indicated that it was probably a glycoprotein, the product of pronase digestion having a molecular weight of about 2000 and properties consistent with those of a carbohydrate (Dukelow, Chernoff & Williams, 1966, 1967; Williams, Abney, Chernoff, Dukelow & Pinsker, 1967; Hunter & Nornes, 1969). Decapacitation factor was removed from solution when capacitated spermatozoa were added, which suggested that receptors for glycoproteins exist on the sperm surface, and is also reported to have enzyme-inhibiting powers, though only on the so-called corona-penetration enzyme (Williams, 1974).

Another theme in the capacitation story began when Yanagimachi & Chang (1964) reported that hamster epididymal spermatozoa could undergo capacita-
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...tion in vitro if they were incubated with egg and cumulus masses accompanied by oviduct secretions. The system was simplified by Barros & Austin (1967) who found that hamster epididymal spermatozoa would become capacitated and take part in fertilization when incubated with immediately prevulatory hamster oocytes accompanied by follicular fluid. Secretions of the female tract were thus shown to be inessential for capacitation. In addition, spermatozoa incubated for about 4 hr in the presence of follicle contents could proceed without delay to take part in fertilization when transferred to a fresh suspension of oocytes. The spermatozoa exhibited acrosome reactions synchronously with the attainment of the ability to penetrate eggs. Bavister (1971, 1973) then showed that capacitation of hamster spermatozoa could take place in a medium containing crystalline bovine serum albumen but neither tract secretions nor follicle contents. Spermatozoa would survive for >12 hr in this medium but would not evince an acrosome reaction; the reaction could be evoked promptly at any time after incubation for 4 hr if spermatozoa were transferred to media containing eggs with their follicular cell investments. These observations showed clearly that capacitation and the acrosome reaction are separate processes, with the former a necessary precursor to the latter. Subsequent work (Austin, Bavister & Edwards, 1973) demonstrated that serum albumen as a macromolecular component could be replaced by polyvinylpyrrolidone, though results were very irregular (better prospects are offered by dextran, B. D. Bavister, personal communication). It could then be inferred that capacitation of hamster spermatozoa was in the nature of an endogenous change promoted by maintenance in appropriate media which need not contain any contribution from the female organism (Austin & Bavister, 1975).

A different line of investigation was followed by Yanagimachi (1969a, b) who fractionated bovine follicular fluid and tested the products on hamster spermatozoa. The fluid contained two components of importance—a dialysable, heat-stable compound which strongly favoured sperm motility and a nondialysable heat-labile component, both being required for capacitation. The development of a distinctive 'frenzied' whip-lash motility seemed to mark the completion of capacitation. Blood serum (heated to destroy complement) was later shown to contain two factors with the same properties as those in follicular fluid (Yanagimachi, 1970a; Barros & Garavagno, 1970). These workers thus also obtained capacitation in the absence of female tract secretions or follicle contents. In this research, the completion of capacitation was recognized by the occurrence of the acrosome reaction, and the investigators concerned did not initially distinguish between the two processes. A reasonable interpretation, however, is that the observations are consistent with the inferences drawn in the previous paragraph, and that the motility-promoting factor permits the occurrence of capacitation, while the non-dialysable heat-labile component specifically triggers the acrosome reaction. It should be cautioned, though, that the true position may not be as simple as this.

Gwatkin & Hutchison (1971) maintained that artificial media to which β-glucuronidase was added would favour the development of capacitation, followed by the acrosome reaction, if eggs and cumulus cells were present; the effect of enzyme addition could be inhibited by adding the specific β-glucuronidase...
dase inhibitor glucaro-(1→4)-lactone. More recently, R. B. L. Gwatkin and C. F. Hutchison (personal communication) have been able to obtain capacitation of hamster spermatozoa by incubating them in the supernatant from the centrifugation of epididymal secretions—results apparently in conflict with those obtained in research on decapacitation factor. This effect of epididymal secretions could be inhibited by glucaro-(1→4)-lactone. B. D. Bavister (personal communication) has also noted that either epididymal secretions or the supernatant from a dense sperm suspension could provide an appropriate medium for capacitation.

Oliphant & Brackett (1973a) have shown that capacitation of mouse spermatozoa can be achieved in vitro by subjecting them to a hypertonic medium, which evidently removed protein material from the sperm surface, and that decapacitation could also be demonstrated. The same workers (1973b) have found, too, that the ability of antibodies against sperm-surface antigen to agglutinate rabbit spermatozoa diminished with the duration of incubation of the spermatozoa in the uterus; in addition, the uptake of 14C-labelled antibodies by washed ejaculated spermatozoa decreased progressively with incubation in uterine fluid, but was not affected by incubation in artificial medium for the same period. Brackett (1974) also reports that rabbit spermatozoa, like those of mice, can now be capacitated in vitro by incubation in a hypertonic medium.

The research described in the last four paragraphs supports the idea that the important feature of a medium promoting capacitation is that it allows full normal motility for a sufficient length of time; if these circumstances are provided, capacitation can be thought to occur as the result of endogenous changes. Short cuts can be introduced by including glycolytic agents in the medium or by using hypertonic media. Perhaps, in normal circumstances, the spermatozoon will generate its own glycolytic agent and thus divest itself of its glycoprotein extrinsic stabilizing coat.

THE ACROSOME REACTION

The acrosome reactions of certain invertebrate spermatozoa were initially studied during the early 1950s (see Dan, 1967) and a few years later the mammalian acrosome reaction was recognized (Austin & Bishop, 1958). Its occurrence, as seen by phase-contrast microscopy, was recorded in the golden and Chinese hamsters, the Libyan jird and the guinea-pig. Although at first erroneously thought to involve loss of the whole acrosome and to be a 'visual concomitant' of capacitation, its significance for the release of acrosomal enzyme (at that time only hyaluronidase was known) was correctly surmised. Spermatozoa with intact acrosomes were found to be incapable of penetrating the cumulus matrix while those 'lacking' acrosomes were able to move freely through this material (Austin, 1960). The idea that passage through the cumulus matrix is made possible by release of acrosomal enzymes is supported by the recent work of Metz, Seiguer & Castro (1972) who showed that anti-sperm antibodies (mostly directed against the acrosomal enzymes) prevent break-up of the cumulus.

Pikó & Tyler (1964) examined rat eggs by electron microscopy at a time
when sperm penetration was taking place, and they found sperm heads apparently devoid of acrosomes just outside the zona pellucida. These sperm heads were accompanied by small accumulations of membranous vesicles, and Pikó & Taylor inferred that these came from the breakdown of the acrosome. More detailed observations were made by Barros, Bedford, Franklin & Austin (1967) and Bedford (1967), and it became clear that the outer acrosome membrane of the spermatozoon underwent fusion (vesiculation) with the overlying plasma membrane, and the complex thus formed became detached from the sperm head. Release of acrosomal contents could reasonably be supposed to take place as soon as apertures were formed in the surrounding membrane barriers. Subsequent work, in which coincidence between the reactions of acrosomes and the start of sperm penetration was observed, has been described above.

Vesiculation of sperm cell membranes is not restricted to the events normally succeeding capacitation and leading to penetration of the egg. When spermatozoa are treated with strongly hypotonic media, unheated serum, detergents and certain other agents, membranes may well undergo vesiculation, and sometimes the overall effect resembles an acrosome reaction. The changes are, however, commonly associated with cell death and are very unlikely to (but do occasionally) include preservation of membrane continuity about the cell. The ‘true’ acrosome reaction is associated with cell survival and this criterion aids distinction between degenerative and ‘physiological’ membrane changes. The ‘true’ reaction seems indeed to be accompanied by a striking increase in the vigour and amplitude of the sperm tail beat (Yanagimachi, 1970b; Barros, Berrios & Herrera, 1973). ‘True’ reactions can evidently be induced in some instances without capacitation: unheated serum induced acrosome reactions in guinea-pig spermatozoa in about 10 min, the cells maintaining strong motility for several minutes thereafter (M. H. Johnson, personal communication); guinea-pig spermatozoa were also found to develop acrosome reactions (with survival) in about 10 min if held in a balanced salt solution on a glass slide under a coverslip (Barros et al., 1973).

The occurrence of ‘false’ and accelerated reactions in no way detracts from the validity of the theory that, in normal circumstances, the acrosome reaction cannot occur until capacitation has been completed and is then provoked by a specific stimulus. The nature and origin of the specific stimulus are still matters for debate. Yanagimachi (1969b) believes that the nondialysable component which probably provokes the hamster acrosome reaction originates from the oocyte or the follicle cells investing it, and Pavlok & McLaren (1972), working with the mouse, essentially agreed. The likelihood that the follicular cells are implicated was reduced when Fraser, Dandekar & Vaidya (1971) found that denuded rabbit eggs were fertilizable at the same frequency in vitro as eggs with coronas or intact cumuli, though the possibility that traces of cumulus matrix remained about these eggs would have been difficult to exclude. The egg itself therefore looks like being the source of the stimulus for the acrosome reaction, but the agent could also originate elsewhere, be carried in the blood stream, pass into the follicle and be taken up by the egg and its investments. Interest in Yanagimachi’s nondialysable serum component should clearly be maintained, and the recent work of Bavister & Morton (1974) on the fraction-
ation of serum protein components could lead to identification of the effective molecule(s).

In contrast to all that has been said about the release of hyaluronidase in the acrosome reaction, in the last year or two there have appeared reports on observations which indicate that, under specified conditions in vitro, hyaluronidase is released from hamster (but not guinea-pig) spermatozoa well before the occurrence of the acrosome reaction (see Talbot & Franklin, 1974, who also discuss the earlier papers). The mechanism of this release and its significance for ideas about capacitation have yet to be elucidated.

To begin with, as mentioned above, the only enzyme thought to be present in and released from the acrosome was hyaluronidase, but detailed biochemical studies, requiring break-up of sperm membranes and differential centrifugation, established the presence also of other lytic enzymes including a trypsin-like enzyme (now often called 'acrosin'), catalase, carbonic anhydrase, lactic dehydrogenase (Stambaugh & Buckley, 1969, 1970), aryl phosphatase, aryl sulphatase, β-N-acetylgalacosaminidase and phospholipase A (Allison & Hartree, 1970). Hyaluronidase seems likely to remain the most important acrosomal enzyme for the spermatozoon traversing the cumulus matrix; it breaks the bonds linking the hyaluronic acid and protein molecules and effectively liquefies the matrix. Antibodies raised specifically against hyaluronidase can inhibit the fertilization of rabbit eggs in vitro (indeed hyaluronidase is the only acrosomal enzyme against which antibodies have proved to be effective inhibitors) (Metz, 1974).

When the vesciculated acrosome–plasma membrane complex has been sloughed and the acrosomal contents dissipated, the sperm head is lined only by inner acrosome membrane anteriorly and the confluent plasma membrane posteriorly. This state of affairs is regularly found in spermatozoa that have reached the surface of the zona pellucida.

Before penetration of the zona pellucida can begin, attachment of the spermatozoon to this investment is necessary. Sperm attachment has only been studied with cumulus-free eggs: with these, it occurs more readily if they are homologous than if heterologous, and more readily if unfertilized than fertilized. But attachment may be an even more complex process than such observations indicate. Hartmann & Gwatkin (1971) adduced evidence that attachment of capacitated mouse spermatozoa showed two phases of 'binding', the first (B1) occurs rapidly (within 2 min of mixing) and is temporary; after about 30 min, a second firmer binding (B2) occurs and is shortly followed by penetration of the zona pellucida. The B1 phase is thought to evoke the release of a factor from the vitellus which terminates this attachment; the factor could be a protease because treatment with the specific protease inhibitor p-aminobenzamidine prolongs B1 (Hartmann & Hutchison, 1974). By contrast, Barros & Yanagimachi (1972) found that when the hamster egg is fertilized in vitro, the spermatozoon spends only about 5 min on the zona surface and then penetrates, reaching the perivitelline space in about 15 min.

The next step in penetration of egg investments involves passage through the zona pellucida, which must be accomplished quite rapidly in view of the rarity with which spermatozoa are seen in the act of penetration. The spermatozoon
follows a curved path through the zona, leaving behind a narrow slit which can be seen for a few hours after penetration (Austin & Bishop, 1958). The means whereby passage is effected are not clear—presumably an agent having a lytic action on the zona must be implicated, but the narrowness of the slit testifies to non-diffusion of this agent from the sperm head. Presumably such a ‘zona lysin’ is intimately associated with the inner acrosome membrane, the molecules possibly being intercalated in the lipid leaflet. By using membrane-disaggregating agents of increasing vigour, Srivastava (1973) has been able to demonstrate a protease associated with the inner acrosome membrane, i.e. distinct from the enzymes in the bulk of the acrosome. Gaddum-Ross (1974) also reports lytic action in the immediate vicinity of the inner acrosome membrane from observations on reacted spermatozoa with the gelatin-plate technique. All these points are consistent with the idea that the inner acrosome membrane in the mammalian spermatozoon has properties that distinguish it from the outer acrosome membrane and from the sperm plasma membrane, which may well account for the failure of the inner acrosome membrane to fuse subsequently with the egg plasma membrane (see below).

GAMETE FUSION

Once through the zona pellucida the sperm head evidently makes immediate contact with the surface of the vitellus and becomes adherent to it. Observations on living material revealed that the spermatozoon virtually ceased its motility possibly within seconds, and certainly within minutes, of its achieving the vitelline surface. Electron microscopic study of such spermatozoa showed that fusion between sperm and egg plasma membranes had already occurred (R. Yanagimachi, personal communication).

The occurrence of membrane fusion in mammalian sperm–egg union was first reported from ultrastructural observation on rat gametes by Szollosi & Ris (1961), who demonstrated clearly that the plasma membranes of the two cells become confluent, forming a single mosaic membrane around the now bineucleate cell. An important distinction was thus established between cell fusion and cell phagocytosis; in the latter process the cytoplasm of the two cells remains separated by the two intact plasma membranes. Essentially the same facts were simultaneously shown to be true for gamete union in the polychaete worm *Hydroides* (Colwin & Colwin, 1961).

Initial fusion between gametes in mammals involves the sperm plasma membrane in specific regions, either over the equatorial part of the acrosome or just posteriorly to this region (rat: Piko & Tyler, 1964; hamster: Barros & Franklin, 1968; Yanagimachi & Noda, 1970a, b; Franklin, 1974; mouse: Stefanini, Oura & Zamboni, 1969; rabbit: Bedford, 1970, 1972). This is in strong contrast to the initial event in marine invertebrates, in which it is the inner acrosome membrane at the tip of the acrosome filament (or filaments, in the case of *Hydroides*) that fuses with the egg plasma membrane (see Colwin & Colwin, 1967). The difference may be attributable in some way to the absence of an acrosome filament in reacted mammalian spermatozoa (see Austin, 1968), but seems likely also to arise from intrinsic differences in the inner acrosome membrane.
itself, possibly due to the incorporation of the postulated zona lytic agent in the membrane. This latter suggestion finds support in observations on the fate of the inner acrosome membrane as fertilization proceeds; it does not fuse with the egg plasma membrane but is engulfed as a separate vesicular structure which gradually disintegrates in the egg cytoplasm (Pikó & Tyler, 1964; Barros & Franklin, 1968; Pikó, 1969; Yanagimachi & Noda, 1970b).

Sperm attachment to and fusion with the vitellus is known to fail under certain specific conditions. The most common is that following breakdown of cortical granules and the establishment of the vitelline block to polyspermy, of which more will be said later. A second condition is the absence of capacitation, which is evidently as much a prerequisite for fusion in zona-free eggs as it is with intact ones (Yanagimachi & Noda, 1970c). Another lies in the existence of an apparent bar to heterospecific fertilization. Many mammalian hybrids are known but others appear to be blocked (see Gray, 1954); in a few instances, the barrier appears to be at the vitelline surface; the occurrence has been recorded of two rat spermatozoa in the perivitelline space of a Mastomys coucha egg (A. W. H. Braden, personal communication) and of a rabbit spermatozoon in a rat egg (Dickmann, 1962). In both these eggs (recovered after artificial insemination) the spermatozoon had failed to become attached to the vitellus. Non-attachment of perivitelline spermatozoa is also known in mouse eggs subjected to heat treatment (Braden & Austin, 1954) and in an inbred strain of mice with a genetic defect (Krzanowska, 1960). In some rabbit eggs unattached perivitelline spermatozoa were seen following insemination of the rabbits with semen treated with an enzyme inhibitor (53 d/K, a condensation product of formaldehyde and hydroquinone) which had the property of becoming firmly adherent to the sperm surface (Parkes, Rogers & Spensley, 1954). From these assorted data it can be inferred that gamete attachment depends on specific receptors, and either the sperm surface or vitelline surface may change in such a way as to preclude attachment. Virtually nothing is known as yet about the molecular biology of gamete attachment.

**BREAKDOWN OF CORTICAL GRANULES**

Cortical granules (vesicles), which disappear after sperm penetration, have been found in all mammalian eggs that have been examined for them, and similar or related structures occur in the eggs of echinoderms, polychaetes, anurans and some fish (see Austin, 1968). In all these instances, material is extruded from the vesicular structures into the perivitelline space in response to sperm attachment to the vitellus, but the function of the released material varies a good deal with species. In mammals, the precise function is uncertain but an obvious possibility is that the released material is responsible for the altered reaction of the zona pellucida after sperm entry to treatment with various enzymes and other agents. Indeed, except in the lagomorphs, permeability of the zona pellucida to spermatozoa is also lost—a change testifying to the occurrence of the ‘zona reaction’ (Braden, Austin & David, 1954). Barros & Yanagimachi (1972) estimate that the zona reaction in the hamster egg is complete within 15 min of sperm attachment to the vitellus. Earlier, the same authors (1971) had demon-
Stratified that material recovered from reacted (penetrated) eggs could apparently render impermeable the zona of unpenetrated eggs when these were treated in vitro. Furthermore, there was no evidence of a zona reaction in aged eggs in which cortical granule breakdown had failed to follow sperm penetration. These observations would seem clearly to indicate a causal relationship between cortical granule breakdown and the zona reaction, and suggest that the extruded material might have a kind of ‘tanning’ action on the zona pellucida, as proposed by Rothschild (1956, p. 115).

Other mechanisms have also been envisaged. Gwatkin, Williams, Hartmann & Kniazuk (1973) offered evidence that the hamster cortical granules released a trypsin-like enzyme, finding that trypsin inhibitors prevented the consequences of cortical granule breakdown. More recently, Gwatkin (1974) reported that the cortical granule protease closely resembles acrosin; taken in conjunction with the derivation of acrosome and cortical granules from Golgi complexes, the observation points to a much closer homology between the two gamete organelles than had previously been appreciated. Some spermatozoa, such as those of nematodes (Foor, 1970), do in fact possess a number of acrosomal vesicles instead of one. It is of interest too that a protease has been detected in sea urchin cortical granules (Vacquier, Epel & Douglas, 1972). Cortical granule protease could act by destroying sperm receptors on the zona as well as on the vitelline surface, and so could be held accountable for both the zona reaction and the block to polyspermy. On the other hand, Conrad, Buckley & Stambaugh (1971) maintained that, in the rabbit, the cortical granules released a protease inhibitor, which could act by interfering with vitelline sperm-receptor action.

Although sperm attachment normally leads to cortical granule breakdown, spontaneous and experimental (cold-shock) activation have been reported not to be associated with this reaction in the hamster (Austin, 1956) and rabbit (Flechon & Thibault, 1964). By contrast, electrical stimulation of hamster eggs with a square-wave pulse of 150 V for 1 msec was said to be highly effective in eliciting the reaction (Gwatkin et al., 1973; Gwatkin & Williams, 1974).

CONCLUSIONS

The observations described in this review support the following tentative conclusions: (1) capacitation involves the removal of a glycoprotein coat from the spermatozoon which is probably deposited in the epididymis and has the function of stabilizing the plasma membrane in the region where it overlies the acrosome; (2) removal of this extraneous coat is brought about by metabolic changes occurring in the spermatozoon, possibly involving the production of an appropriate lytic agent; (3) these endogenous changes depend upon maintenance of the spermatozoon for a sufficient period in a suitable medium, an important property of which is that it permits continued vigorous motility on the part of the spermatozoon; (4) under natural conditions this property is attributable to the presence of a low molecular weight heat-stable component of the female genital tract secretions, but its effect can be mimicked by artificial means; (5) removal of the extraneous coat unblocks the acrosome reaction; (6) induction of the acrosome reaction is due to a specific agent which appears to
emanate from the egg or surrounding cumulus cells; (7) this agent is a high molecular weight heat-labile compound, probably a protein; (8) the acrosome reaction allows the escape of hydrolytic enzymes, including hyaluronidase, which enable the spermatozoon to penetrate the matrix of the cumulus oophorus about the egg; (9) a protease-like enzyme intimately associated with the inner acrosome membrane (the 'zona lysin') permits penetration of the zona pellucida; (10) fusion between the sperm and egg plasma membranes results in release of the contents of cortical granules by fusion of their membranes with the egg plasma membrane; (11) active material in the extruded cortical granule contents either renders the zona pellucida insoluble to the zona lysin or inhibits that enzyme.

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REFERENCES

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