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# The Biology and Chemistry of Fertilization

PAUL M. WASSARMAN

**Fertilization of eggs by sperm, the means by which sexual reproduction takes place in nearly all multicellular organisms, is fundamental to the maintenance of life. In both mammals and nonmammals, the pathway that leads to fusion of an egg with a single sperm consists of many steps that occur in a compulsory order. These steps include species-specific cellular recognition, intracellular and intercellular membrane fusions, and enzyme-catalyzed modifications of cellular investments. In several instances, the molecular mechanisms that underlie these events during mammalian fertilization are beginning to be revealed.**

AS A CONSEQUENCE OF ITS FUNDAMENTAL ROLE IN THE life history of most higher organisms, fertilization has been of great interest to scientists for more than a century. Building upon original contributions of E. Van Beneden, O. Hertwig, and H. Fol, made between 1875 and 1880, we are rapidly expanding our understanding of the biology and chemistry of mammalian fertilization. In recent years this knowledge has influenced both medical and ethical aspects of conception and contraception and undoubtedly will continue to do so (*1*).

I shall describe here some principal cellular and molecular features of fertilization in mammals, from the initial encounter of sperm and egg to their fusion with one another to form a zygote. Although much of the discussion is drawn from in vitro experiments with mouse gametes, it is likely that the stratagem for fertilization described applies to in vivo fertilization for most mammals, including humans. Throughout the article, fertilization in mice is compared with fertilization in sea urchins, the most extensively studied nonmammalian species. Such comparisons illustrate the large number of cellular and molecular features common to fertilization in these two organisms.

This article is not intended to be comprehensive, but should serve only as an introduction to certain aspects of contemporary mamma-

The author is chairman of the Department of Cell Biology at the Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

lian fertilization research. In the interest of brevity, only work with mouse gametes is presented, many important contributions are not cited, and specific points of view are adopted on issues that may be the subject of some controversy among workers in the field. In view of these shortcomings, and to gain a broader, more historical perspective of the field, it is hoped that readers will refer to more detailed accounts of this subject (*2–10*).

## Chronology of Fertilization

The pathway leading to fertilization of mouse eggs consists of several steps that occur in a compulsory order (Fig. 1). This pathway is derived from studies carried out by many investigators (*2–10*). The following briefly describes steps of this pathway and introduces some relevant terminology.

1) Sperm first associate with ovulated eggs at the surface of the egg's thick extracellular coat, the zona pellucida. This is a relatively loose, nonspecific association between sperm and egg, referred to as attachment.

2) Attached sperm can then form a relatively tenacious, species-specific association with eggs, a state of adhesion referred to as binding. Mutual orientation of gametes during the attachment phase probably influences whether or not sperm-egg interaction progresses to the binding stage. At very high sperm concentrations in vitro, as many as 1500 sperm can bind to a single mouse egg. Sperm bind to zonae pellucidae through plasma membrane overlying the anterior region of the sperm head. Binding is mediated by sperm receptors present in zonae pellucidae and complementary egg-binding proteins present in sperm plasma membrane.

3) Bound sperm then complete the acrosome reaction in preparation for penetration of the zona pellucida and fusion with egg plasma membrane. The acrosome is a lysosome-like organelle, located in the sperm head, overlying the nucleus. The acrosome reaction involves fusion of outer acrosomal membrane and sperm plasma membrane at many sites, resulting in release of small, hybrid vesicles from the anterior region of the sperm head and exposure of inner acrosomal membrane.

4) Acrosome-reacted sperm can then penetrate the zona pellucida, apparently by using a trypsin-like proteinase, acrosin, that is associated with inner acrosomal membrane. Sperm cross the zona pellucida at a rate of about 1 μm per minute, leaving behind an extremely narrow trail, about the diameter of the sperm head.

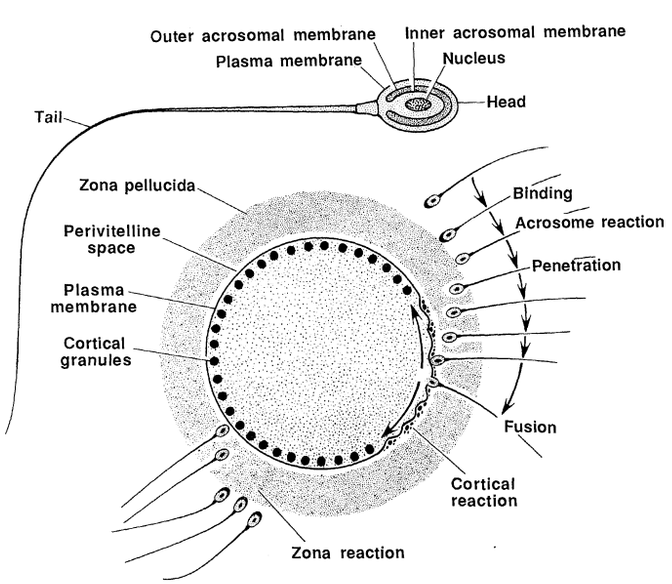


**Fig. 1.** Diagrammatic representation of mouse gametes and the fertilization pathway in mice. The sequence of events includes attachment, followed by binding of sperm to eggs, completion of the acrosome reaction, penetration, sperm-egg fusion, cortical reaction, and zona reaction.

5) Sperm that reach the space between the zona pellucida and egg plasma membrane (perivitelline space) can then fuse with the egg (fertilization). Fusion occurs between plasma membrane overlying the posterior region of the sperm head and egg plasma membrane. Under normal conditions, fertilization of an egg by a single sperm precludes fusion of additional sperm with egg plasma membrane, thus avoiding a lethal condition called polyspermy. This fast block to polyspermy probably involves a transient depolarization of egg plasma membrane immediately after sperm-egg fusion.

6) Fertilization by a single sperm activates the egg, and embryonic development ensues. Fertilization also induces the cortical reaction which, in turn, induces the zona reaction. Cortical granules are small, membrane-bound, lysosome-like organelles that occupy a region of egg cytoplasm just beneath the plasma membrane. Mouse eggs contain several thousand cortical granules. The cortical reaction involves fusion of cortical granule membrane with egg plasma membrane. This fusion results in release of cortical granule contents, including various enzymes, into the perivitelline space. These contents apparently enter the zona pellucida and are responsible for inducing the zona reaction some minutes after fertilization. The zona reaction consists of a general "hardening" of the zona pellucida, as well as loss of its ability to bind sperm; these changes are thought to constitute a slow block to polyspermy.

The pathway just described includes three different membrane fusion events: the acrosome reaction (sperm), fusion of sperm and egg, and the cortical reaction (egg). Two of these events, the acrosome and cortical reactions, involve fusion of plasma membrane with membrane of lysosome-like organelles, the acrosome and cortical granules, respectively. In both instances, the object of membrane fusion is to expose the zona pellucida to contents of the organelle—in one case to enable sperm to penetrate the zona pellucida of unfertilized eggs, and in the other to prevent sperm from penetrating the zona pellucida of fertilized eggs.

The similarity between this pathway and the fertilization pathway for echinoderms (for example, sea urchins) should be mentioned. Despite the evolution from external to internal fertilization, estimated to have occurred over a period of 100 million years or so, many of the cellular and molecular mechanisms involved remain the same. Perhaps this is to be expected, since the objectives of the fertilization process, as well as the participants in the process (sperm and egg), are similar for mice and sea urchins. Some of the common mechanisms are described below.

## Sperm Receptors

For some time it has been known that, among mammals, the egg zona pellucida plays a significant role in restricting interspecific fertilization (*4, 11–13*) (Fig. 2). For example, under in vitro conditions that permit fertilization of eggs by sperm from a homologous species, fertilization of eggs by sperm from a heterologous species rarely occurs unless the zona pellucida is first removed from the eggs. This observation supports the idea that binding of sperm to eggs is mediated by species-specific sperm receptors present in zonae pellucidae. Therefore, the inability of sperm to bind to the zona pellucida of fertilized eggs could possibly be ascribed to inactivation of these sperm receptors after fertilization. Such a situation is analogous to that described for sea urchins, where species-specific binding of sperm to eggs is attributable to sperm receptors present in one of the egg's extracellular coats, the vitelline

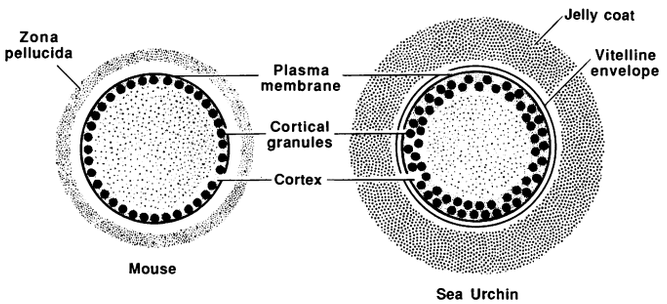


**Fig. 2.** Diagrammatic representation of mouse and sea urchin eggs. [From figure 1 of (*13*), courtesy of Wiley-Interscience]

envelope. After fertilization, the vitelline envelope (Fig. 2) is converted by cortical granule contents into a fertilization envelope, to which sperm are unable to bind.

Recently, the mouse sperm receptor has been identified, isolated, and characterized (*14–16*). The receptor, called ZP3 (molecular weight 83,000), is one of three different glycoproteins that constitute the egg zona pellucida. ZP3 consists of a 44,000-dalton polypeptide chain, to which several asparagine-linked (*N*-linked) and serine-threonine–linked (*O*-linked) oligosaccharides are covalently attached (*16, 17*). Each zona pellucida contains approximately a billion copies of ZP3 that are synthesized and secreted, along with two other zona pellucida glycoproteins (ZP1 and ZP2), by growing ovarian oocytes. During this 2- to 3-week growth period, the three glycoproteins assemble into long, interconnected filaments (*16, 18*), giving rise to a relatively porous zona pellucida, about 7 μm thick, that surrounds fully grown oocytes. At ovulation, fully grown oocytes are transformed into unfertilized eggs and deposited into the oviducts in anticipation of fertilization. The zona pellucida surrounds embryos until the expanded blastocyst stage of development (approximately 100 cells), at which time embryos hatch from their zona pellucida and implant in the uterus.

As a prelude to fertilization, each mouse sperm with an intact acrosome binds to tens of thousands of copies of ZP3 at the outer margin of the egg zona pellucida (*14–16*) (Figs. 3 and 4). To do so, sperm must recognize some molecular features of ZP3 in a species-specific manner. In this context, the ability of ZP3 to function as a sperm receptor is attributable, not to its polypeptide chain, but solely to certain of its *O*-linked oligosaccharides that have an apparent molecular weight of 3900 (*16, 19*). Chemical removal of these oligosaccharides from ZP3 completely destroys its ability to serve as a sperm receptor, although the released oligosaccharides themselves retain many characteristics of a sperm receptor. For example, exposure of sperm to these oligosaccharides, present at only nanomolar concentrations, prevents the sperm from binding to and fertilizing ovulated eggs in vitro. Other oligosaccharides, derived from a wide variety of glycoproteins, including zona pellucida glycoproteins ZP1 and ZP2, have no effect on either sperm binding to or fertilization of ovulated eggs in vitro. These findings may explain why various lectins, monosaccharides, and glycoconjugates prevent binding of sperm to mammalian eggs (*20, 21*). They also may explain why ZP3 continues to function as a sperm receptor in vitro, even after exposure to detergents, denaturants, or high temperatures (*16*).

ZP3 illustrates a role for carbohydrates in supporting specific interactions between eukaryotic cells, in this case, between sperm and egg. A strong case can be made for involvement of carbohydrates in species-specific binding of sperm to eggs in sea urchins as well. Whether ZP3, or *O*-linked oligosaccharides derived from ZP3, exhibit any species specificity has not yet been determined. The great diversity of known oligosaccharide structures is compatible with generation of species specificity for mammalian sperm receptors. The variety of compositions, sequences, branching patterns, conformations, and other features of oligosaccharide structure provide for a staggering number of combinatorial possibilities (*22*). It will be of considerable interest to compare the structures of these ZP3 oligosaccharides with functionally analogous oligosaccharides isolated from eggs of other mammals.

## Egg-Binding Proteins

In sea urchins, glycoprotein sperm receptors are present in the egg vitelline envelope and have a molecular weight greater than 10 million (*23, 24*). These receptors are recognized by bindin, a major protein component of the sperm's acrosome (*24, 25*). Bindin is a 30,500-dalton hydrophobic protein that can be considered a lectin since it recognizes and binds to specific sequences of sugar residues. Therefore, as in mice, sperm receptor oligosaccharides play a primary role in species-specific binding of sea urchin sperm to eggs. Bindin is localized within the acrosome and intimately associated with inner acrosomal membrane. Consequently, it can interact with sperm receptors in the vitelline envelope only after completion of the acrosome reaction. This mode of interaction represents a significant difference between the fertilization pathways of mice and sea urchins, since in mice only acrosome-intact sperm bind to eggs.

In mice, several different kinds of proteins are being considered as candidates for the role of egg-binding protein, played by bindin in sea urchins. These proteins include lectins, glycosyl transferases, proteinases, and glycosidases. In each case, the protein is associated with plasma membrane surrounding the sperm head. For example, galactosyl transferase, an enzyme that normally catalyzes transfer of galactose from uridine 5′-diphosphate–galactose to terminal *N*-

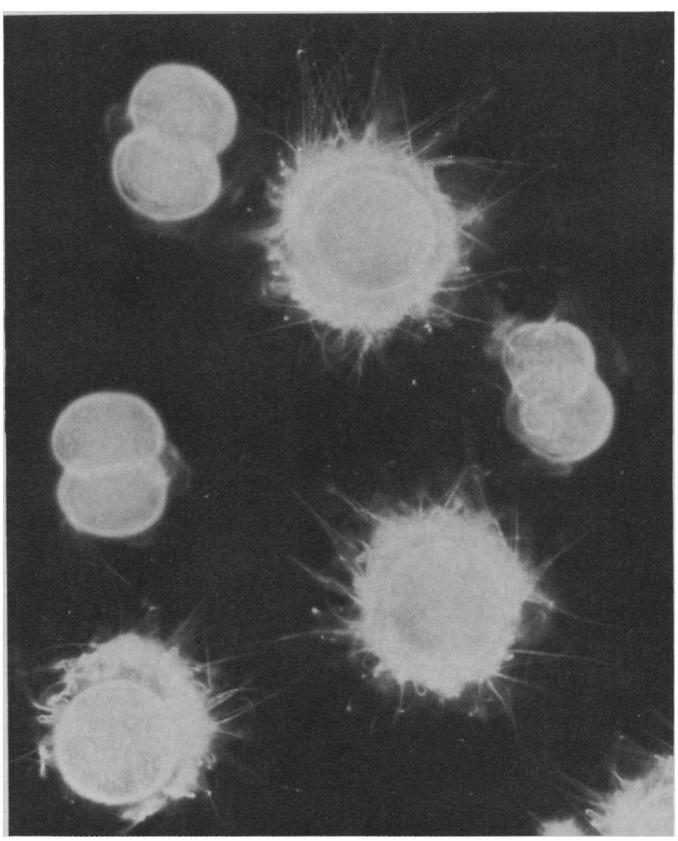

**Fig. 3.** Photomicrograph illustrating the binding of mouse sperm to unfertilized mouse eggs, but not to two-cell mouse embryos in vitro.

acetylglucosamine residues to form *N*-acetyllactosamine, is a potential mediator of sperm binding to eggs (*21, 26*). Presumably, the enzyme recognizes and binds to specific *N*-acetylglucosamine residues on ZP3, a situation consistent with the proposed role of ZP3 *O*-linked oligosaccharides in sperm-egg interaction. Similarly, certain evidence suggests that a trypsin-like proteinase may be involved in binding of sperm to ZP3 (*27*). As in the case of galactosyl transferase, recognition and binding would be accomplished through formation of an enzyme-substrate complex, in which ZP3 serves as substrate. Complex formation must result, not only in binding, but in induction of the acrosome reaction as well. Furthermore, whatever the precise nature of the egg-binding protein, its structure must change in concert with that of the sperm receptor during evolution to provide species-specific binding of sperm to eggs. A more detailed understanding of these aspects of sperm-egg interaction in mice awaits isolation and characterization of the actual egg-binding protein.

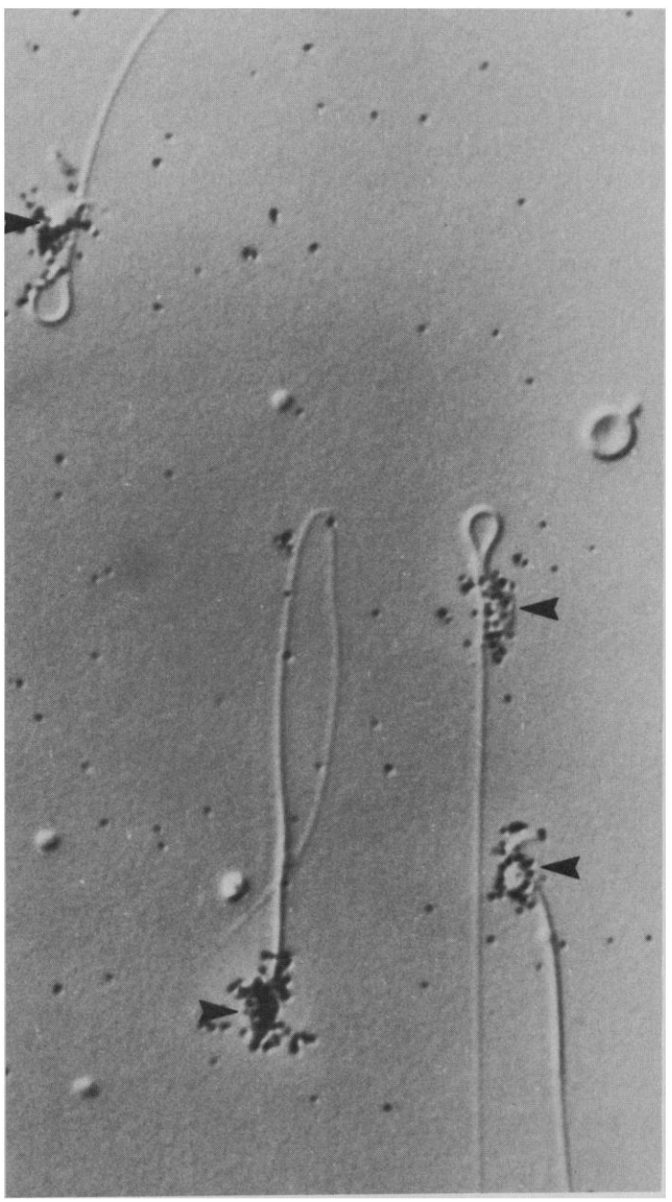

**Fig. 4.** Autoradiographic visualization of radioiodinated mouse sperm receptor (ZP3) bound to acrosome-intact mouse sperm. Arrowheads indicate silver grains localized to the sperm head. [From figure 4 of (*15*), courtesy of Rockefeller University Press]

## Acrosome Reaction

The acrosome is a membrane-bound, lysosome-like organelle that occupies the anterior region of the sperm head, just above the nucleus and beneath the plasma membrane (*28*). The acrosome first appears during transformation of spermatids into spermatozoa (spermiogenesis) as a product of the Golgi complex. A variety of enzymes, including proteinases, glycosidases, phosphatases, arylsulfatases, and phospholipases are present in acrosomes, and some of these may be integral components of inner acrosomal membrane.

For bound sperm to penetrate the zona pellucida and fuse with plasma membrane, they must first complete the acrosome reaction (*29*) (Fig. 5). This reaction may be considered an exocytotic event, analogous overall to somatic cell exocrine secretion from secretory granules. The acrosome reaction involves fusion and vesiculation of sperm plasma and outer acrosomal membranes and results in release of acrosomal contents, as well as exposure of inner acrosomal membrane with its associated enzymes. The acrosome reaction is characterized by $Na^+$ and $Ca^{2+}$ influx and $H^+$ efflux through plasma membrane surrounding the sperm head; the latter involves an adenosine 5′-triphosphate–dependent $H^+$-pump and leads to an increase in intracellular *p*H. There is an absolute requirement for extracellular $Ca^{2+}$, and a $Ca^{2+}$-binding protein, called calmodulin, present in that region of the sperm head between the plasma and outer acrosomal membranes participates in the reaction. In this context, ionophore A23187, an antibiotic that selectively abolishes permeability of plasma membrane to $Ca^{2+}$ and increases cytoplasmic free $Ca^{2+}$, induces both mouse and sea urchin sperm to undergo the acrosome reaction in vitro (Fig. 6). Finally, there are good reasons to believe that, as proposed for somatic cell membrane fusions, phospholipases, as well as lysophospholipids, play a role in fusion of sperm plasma and outer acrosomal membranes.

Since mouse sperm complete the acrosome reaction after binding to the zona pellucida, it is to be expected that a zona pellucida component induces the reaction. In fact, sperm exposed to solubilized egg zonae pellucidae undergo the acrosome reaction in vitro (*30*). This situation is analogous to one described for sea urchins (*31*). In that case, sperm exposed to solubilized egg jelly coats, a thick (30 μm) layer composed of sialoprotein and fucose sulfate polysaccharide that surrounds the vitelline envelope, undergo the acrosome reaction in vitro. In sea urchins, the fucose sulfate polysaccharide induces the acrosome reaction.

Recently, ZP3 has been identified as the acrosome reaction–inducer present in solubilized egg zonae pellucidae (*16*). Purified ZP3, at nanomolar concentrations, is as effective as ionophore A23187 in inducing sperm to undergo the acrosome reaction in vitro. This finding suggests that binding of acrosome-intact sperm to ZP3, through plasma membrane overlying the anterior region of the sperm head, is sufficient to alter the affected plasma membrane so that it becomes capable of fusing with outer acrosomal membrane. In view of the role of $Ca^{2+}$ and the effect of ionophore A23187, it is likely that binding of ZP3 to the sperm head affects ion permeability of the plasma membrane.

Whereas the ability of ZP3 to serve as sperm receptor depends solely on its oligosaccharides, the ability of ZP3 to serve as acrosome reaction–inducer depends on its polypeptide chain as well (*16, 19*). Thus, small glycopeptides and *O*-linked oligosaccharides derived from ZP3 are able to bind to the head of acrosome-intact sperm and prevent them from binding to unfertilized eggs, but neither the glycopeptides nor *O*-linked oligosaccharides can induce sperm to undergo the acrosome reaction. In view of this behavior, it is tempting to suggest that sperm bind to multiple *O*-linked oligosaccharides on each ZP3 molecule (that is, a multivalent interaction), and these oligosaccharides must be joined by polypeptide chain for

the acrosome reaction to be induced. Whether the ZP3 polypeptide chain itself functions as a fusigenic agent remains to be determined.

Finally, mouse sperm that bind to zonae pellucidae and subsequently complete the acrosome reaction can remain bound. This is in marked contrast to the behavior of free-swimming, acrosome-reacted sperm that are unable to bind to zonae pellucidae in vitro (*30*). In this context, recent evidence suggests that another zona pellucida glycoprotein, ZP2 (which has a molecular weight of 120,000), acts as a secondary receptor for bound sperm after the ZP3-induced acrosome reaction (*15*). If ZP2 serves such a function, the question arises as to why free-swimming sperm that have undergone the acrosome reaction are unable to bind to zonae pellucidae. Although the answer is not completely clear, it is likely that the strength of the interaction between ZP2 and acrosome-reacted sperm is insufficient to bind free-swimming sperm, but sufficient to maintain binding of sperm already associated with zonae pellucidae. Since bound, acrosome-reacted sperm must penetrate the zona pellucida, relatively weak interactions between acrosome-reacted sperm and zonae pellucidae would be advantageous for the sperm's progress through the extracellular coat.

Overall, these observations suggest that ZP3 has evolved to discharge the functions of polysaccharide located in the jelly coat (acrosome reaction–inducer) and glycoprotein located in the vitelline envelope (species-specific sperm receptor) of sea urchin eggs. The dual function of ZP3, as sperm receptor and acrosome reaction–inducer, is consistent with the presence of one, rather than two, extracellular coats around mammalian eggs.

## Sperm Penetration

In sea urchins, acrosome-reacted sperm extend a long, membrane-bound process (acrosomal process) that penetrates the jelly coat and attaches by bindin to the vitelline envelope (*9, 29, 31*). On the other hand, mouse sperm that have undergone the acrosome reaction do not extend such a process and must make their way through, or penetrate, the zona pellucida to reach and fuse with egg plasma membrane. Apparently, penetration is accomplished by limited proteolysis of zona pellucida in front of advancing, motile sperm (*4, 32*). A trypsin-like proteinase, called acrosin, is thought to participate in this process, although other sperm proteinases may be involved as well. Acrosin is produced by specific proteolysis of an inactive proenzyme, proacrosin, and may be intimately associated with inner acrosomal membrane. The latter is particularly relevant because advancing sperm leave behind an extremely narrow trail in the zona pellucida, only about the diameter of a sperm head. This observation seems to rule out an extensive release of proteinases by sperm into the zona pellucida. Finally, the need for proteolysis during sperm penetration has been questioned in view of the substantial propulsive force generated by sperm (*33*). Whether such force alone is sufficient to permit sperm to penetrate the zona pellucida remains controversial.

## Sperm-Egg Fusion

Once through the zona pellucida, sperm can make contact with, adhere to, and fuse with (that is, fertilize) an egg. As compared with binding of sperm to zonae pellucidae, fusion of sperm and egg is not particularly species specific (*4, 34*). In mice, plasma membrane at the posterior region of the sperm head fuses with egg plasma membrane; this is a clear departure from the situation in sea urchins, where inner acrosomal membrane at the apical region of the acrosomal process fuses with egg plasma membrane (Fig. 7). In this

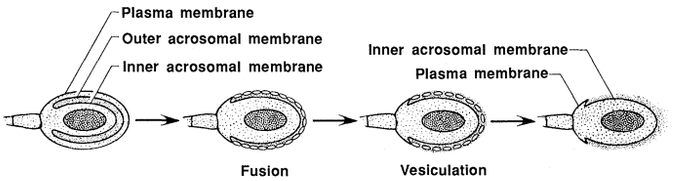


**Fig. 5.** Diagrammatic representation of mouse sperm undergoing the acrosome reaction. [From figure 6 of (*13*), courtesy of Wiley-Interscience]

context, only acrosome-reacted, never acrosome-intact, mouse sperm fuse with eggs freed of their zona pellucida in vitro. Apparently, as a result of the acrosome reaction, plasma membrane that remains associated with the sperm head undergoes changes that allow it to fuse with other membranes (*12, 34*). As proposed for analogous biological systems, localized dehydration at the site of membrane contact and establishment of hydrophobic interactions are probably critical steps in the fusion of sperm and egg plasma membranes (*35*).

Once fused with egg plasma membrane, how does a sperm enter the egg? The surface of both mouse and sea urchin eggs is covered with thousands of plasma membrane–bound projections, called microvilli. Evidence in sea urchins suggests that, after membrane fusion, a group of elongated microvilli cluster tightly around and interdigitate over the sperm head (*36*). As these microvilli are resorbed, the sperm is drawn into the egg. Therefore, sperm

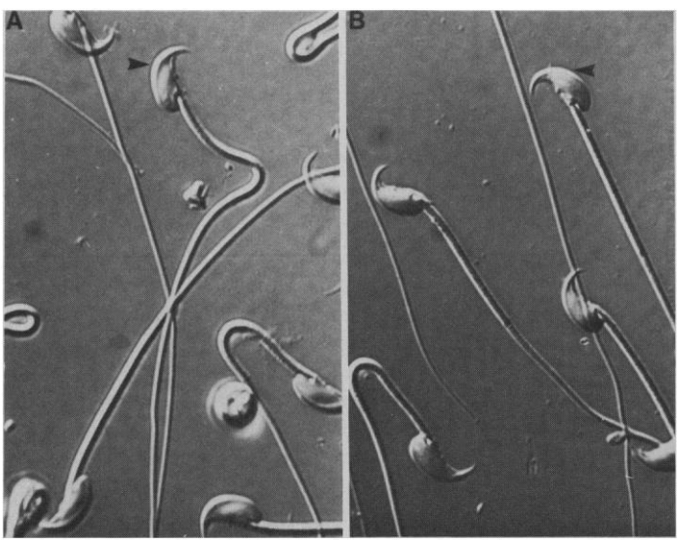


**Fig. 6.** Identification of acrosome-intact (**A**) and acrosome-reacted (**B**) mouse sperm by Nomarski differential-interference contrast microscopy. Arrowheads indicate the presence (A) or absence (B) of an intact acrosome. [From figure 2 of (*15*), courtesy of Rockefeller University Press]

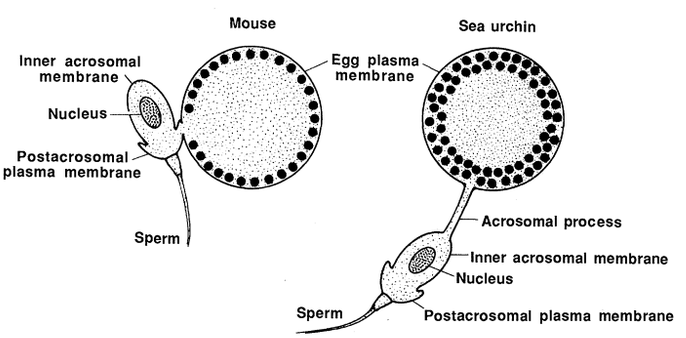


**Fig. 7.** Diagrammatic representation of sperm-egg fusion in mice and sea urchins. [From figure 8 of (*13*), courtesy of Wiley-Interscience]

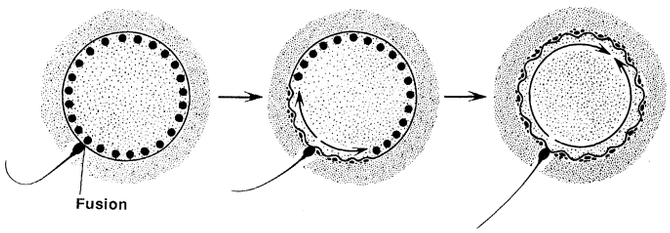


**Fig. 8.** Diagrammatic representation of the cortical reaction following sperm-egg fusion in mice. [From figure 10 of (*13*), courtesy of Wiley-Interscience]

motility, which ceases at the time of fusion in both sea urchins and mice, is not required for sperm entry. Apparently, the driving force for engulfment of a fused sperm comes from a region of cytoplasm just beneath an egg's plasma membrane (within 2 μm), the so-called cortex of the egg. The cortex contains contractile proteins, actin and myosin, and undergoes contraction at fertilization. Since bundles of actin filaments extend from microvilli into the cortex, such contraction probably draws both microvilli and an associated sperm into egg cytoplasm.

Within the first minute after fusion of an egg with a single sperm, several changes in the ionic composition of the egg occur. These changes, recognized primarily from studies of sea urchin fertilization, serve as signals for both activation of eggs and prevention of polyspermy. Although discussion of the former subject is beyond the scope of this article (*37, 38*), certain aspects of the latter are discussed below.

## Fast Block to Polyspermy

Sea urchin eggs undergo a relatively large depolarization within about 3 seconds of fertilization (*39*). The change in potential, which results from an altered membrane permeability to certain ions, can be as much as 80 mV and lasts as long as 1 minute before repolarization. This rapid depolarization of egg plasma membrane after fertilization provides a fast block to polyspermy, which is transient. Conversion of the vitelline envelope into a fertilization envelope occurs within about 1 minute of fertilization, and provides a slow block to polyspermy, which is permanent (*40*).

Whether mouse eggs also exhibit an electrically mediated, fast block to polyspermy in response to fertilization is unclear from available evidence (*41*). Although changes in membrane potential have been noted, the patterns and magnitudes of these changes do not resemble those observed with sea urchin eggs. Since very few mouse sperm reach the perivitelline space in vivo, the necessity for a fast block to polyspermy is certainly diminished as compared with sea urchins. However, this teleological argument and the available evidence do not exclude the possibility of an electrical block to polyspermy in mice and other mammals.

## Cortical Reaction

Cortical granules first appear during growth of mouse oocytes as products of the oocyte's extensive Golgi complex (*42*). Cortical granules increase in number as oocytes increase in diameter. These membrane-bound, lysosome-like organelles are from 200 to 600 nm in diameter and, for the most part, occupy the egg cortex. Each unfertilized mouse egg has approximately 4,000 cortical granules, a modest number compared with the 15,000 or so cortical granules found in sea urchin eggs.

In both mice and sea urchins, the cortical reaction involves fusion of plasma and cortical granule membranes at about the time of fertilization (*38, 42*) (Fig. 8). Membrane fusion is propagated as a wave, emanating from the point of sperm-egg fusion, over the entire egg surface. Apparently, release of $Ca^{2+}$ from cytoplasmic stores is responsible for propagation of a wave around a recently fertilized egg. During this period, the concentration of free $Ca^{2+}$ in the cortex may exceed 1 μ*M* to 5 μ*M*. In this context, ionophore A23187 induces unfertilized eggs to undergo the cortical reaction in vitro, just as it induces sperm to undergo the acrosome reaction. The cortical reaction leads to a transient increase in egg surface area, characterized primarily by elongation of thousands of microvilli at the egg surface and, perhaps, by reorganization of plasma membrane.

As a result of the cortical reaction, cortical granule contents are deposited into the perivitelline space between the plasma membrane and zona pellucida (*4, 38, 42*). As expected for a lysosome-like organelle, the contents include various hydrolytic enzymes, such as proteinases, and peroxidases. Since the zona pellucida is highly porous, making it permeable to large macromolecules and even small viruses, these enzymes can enter the zona pellucida and modify its constituents (that is, induce the zona reaction). Once again, this situation is analogous in many ways to that described for sea urchins. The cortical granule contents of sea urchin eggs include enzymes, structural proteins, and mucopolysaccharides, which together with the vitelline envelope inactivate sperm receptors and form a fertilization envelope. These events help to prevent polyspermy and provide a protective layer around the zygote and cleavage stage embryo.

## Zona Reaction

Apparently, changes that zonae pellucidae undergo during the zona reaction in mice are more subtle than those that accompany the formation of the fertilization envelope in sea urchins. In both cases, changes are brought about by egg cortical granule components in response to fertilization or parthenogenetic activation (for example, by ionophore A23187) and manifested as hardening of the extracellular layer and inactivation of sperm receptors (*38, 42*). The former change makes the extracellular coat callous and refractory to sperm penetration, and the latter prevents sperm binding to fertilized eggs, together constituting a slow block to polyspermy. However, whereas the formation of the fertilization envelope in sea urchins is characterized by extensive ultrastructural reorganization of the vitelline envelope (*40*), the zona pellucida of fertilized mouse eggs resembles that of unfertilized egg zonae pellucidae.

ZP3 isolated from fertilized egg zonae pellucidae, called $ZP3_f$, does not behave as either a sperm receptor or an acrosome reaction–inducer in vitro (*13, 16*). These findings are consistent with the failure of sperm to bind to the zona pellucida of fertilized eggs or embryos (Fig. 3). Therefore, it is reasonable to assume that, shortly after sperm-egg fusion in mice, ZP3 is altered so that it becomes incapable of performing its biological functions. Conversion of ZP3 to $ZP3_f$ also could explain why sperm fail to bind to the zona pellucida of unfertilized eggs exposed to cortical granule exudate in vitro (*43*).

Whatever the molecular nature of the alteration to ZP3 after fertilization, it does not result in a detectable change in the glycoprotein's molecular weight or isoelectric point (*16*). Although a trypsin-like, cortical granule proteinase has been implicated in inactivation of sea urchin sperm receptors after fertilization (*44*), as yet comparable evidence for proteolysis of ZP3 has not been obtained (*16*). In view of the primary role of *O*-linked oligosaccha-

rides in sperm receptor function of ZP3, the possibility that a cortical granule glycosidase, rather than proteinase, converts ZP3 to $ZP3_f$ should be considered. In this context, preliminary evidence suggests that removal of specific sugar residues from ZP3-derived *O*-linked oligosaccharides can prevent the oligosaccharides from binding to sperm (*45*).

The zona reaction in mice includes not only inactivation of sperm receptors, but a so-called hardening of zonae pellucidae as well (*4, 46*). The zona pellucida of both unfertilized and fertilized eggs can be dissolved in vitro by a variety of agents that either do (for example, proteinases and reducing agents) or do not (for example, heat and low *p*H) disrupt covalent bonds. However, fertilized egg zonae pellucidae are considerably more resistant than unfertilized egg zonae pellucidae to all of these agents. This difference in solubility is thought to reflect structural changes that make the zona pellucida of fertilized eggs refractory to sperm penetration. Consequently, sperm that had partially penetrated the zona pellucida before the zona reaction are prevented from further penetration after the zona reaction.

The fertilization envelope of fertilized sea urchin eggs is also considerably more resistant to solubilizing agents than the vitelline envelope of unfertilized eggs. Hardening of the vitelline envelope is attributable in large measure to cross-linking of tyrosine residues, catalyzed by a cortical granule peroxidase (ovoperoxidase) (*47*). Formation of di- and trityrosyl residues converts individual vitelline envelope and cortical granule proteins, which together constitute the fertilization envelope, into an extensive, covalently linked, protein network that is extremely insoluble.

Do tyrosine cross-links account for hardening of the zona pellucida after fertilization of mouse eggs? Apparently not. Although an ovoperoxidase is released from cortical granules into the perivitelline space during the cortical reaction (*48*), ZP1, ZP2, and ZP3 are not covalently cross-linked to one another in fertilized egg zonae pellucidae (*16, 49*). Agents that disrupt noncovalent interactions between proteins continue to solubilize these zonae pellucidae, albeit under much harsher conditions than those required to solubilize unfertilized egg zonae pellucidae. Furthermore, cross-linked oligomers of ZP1, ZP2, and ZP3 of high molecular weight are not found in solubilized preparations of fertilized egg zonae pellucidae. On the other hand, concomitant with the cortical reaction, ZP2 (molecular weight 120,000) undergoes limited proteolysis, generating one or more small peptides that remain covalently attached to the glycoprotein by intramolecular disulfide bonds (*49*). Treatment of this form of ZP2 with reducing agents releases the small peptide or peptides, generating a 90,000-dalton species, called $ZP2_f$. Whether limited proteolysis of ZP2 alone accounts for hardening of zonae pellucidae after fertilization remains to be determined. Since ZP2 and ZP3 together form the repeating structural unit of zona pellucida filaments (*16, 50*), a change in ZP2 conformation may account for changes in the extracellular coat's physical properties.

## Final Comments

As in many other biological systems (*51*), carbohydrates play a fundamental role in species-specific, sperm-egg recognition and binding during fertilization in mice and sea urchins. This is exemplified by the mouse sperm receptor, ZP3, and sea urchin egg-binding protein, bindin. Similarly, as in other biological systems (*52*), membrane fusion and proteolysis play vital roles before (acrosome reaction and sperm penetration), during, and after (cortical reaction, zona reaction, and fertilization envelope formation) fertilization in mice and sea urchins. Therefore, although fertilization is a highly specialized process, each step in the fertilization pathway has counterparts found elsewhere in cellular biology. Consequently, identification of causative agents and elucidation of their mechanisms of action during fertilization have been aided immeasurably by advances in other areas of biological research on both eukaryotes and prokaryotes.

The prospects for fertilization research are bright. Recombinant DNA and monoclonal antibody technology already have begun to open doors to aspects of fertilization research that hitherto were closed (for example, *53*). Application of these and other contemporary experimental approaches should provide answers to questions about fertilization that biologists have wrestled with for more than a century. There is reason for guarded optimism in considering the ways in which this information may be applied.

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