# The Mating Game: What Happens When Sperm Meets Egg

For most species—at least for those who do not worry about overpopulation—the aim of the mating game is fertilization, the union of sperm and eggs to produce new individuals. Investigators have been studying fertilization for some three centuries, ever since they discovered that there were such entities as sperm and eggs. Over the years they have developed a picture of the sequence of events that occurs during the process.

First, the sperm must be activated, a step that for some species occurs when the sperm contact the outer coating of the egg. Activation is required in order for the sperm to bind to the egg itself. One—and only one—of the bound sperm then penetrates and thus fertilizes the egg. This fertilization step induces changes in the egg that have two major effects. The first is the blockage of fertilization by additional sperm; the second is the switching of the egg from quiescence to the vigorous activity needed for cell division.

This picture is not new. What is new is an understanding of biochemical events involved in each of the steps. Not only are these findings helping to solve the "sweet mystery of life," but they may also provide the basis for the development of improved methods of birth control.

In order to fertilize an egg, a sperm must first bind to it. This binding is often species-specific. The sperm of one sea urchin species, for example, binds to eggs of the same species and not to those of any other, even though eggs and sperm from several species may be present in the same water. One of the recent developments in fertilization research is an understanding of the cause of this specificity. It appears to reside in the mutual recognition between a protein found in the sperm and receptors for the protein that are located on the egg. The sperm protein serves as a bridge to link sperm to eggs of the appropriate species.

Victor Vacquier and his colleagues at the University of California at Davis have isolated a protein from sea urchin sperm that apparently mediates sperm binding to eggs. The protein, which they call bindin, has the appropriate species-specificity. When they isolated bindins from two different species of sea urchins, they found that each one binds to and causes the clumping of eggs of the same species but not any of the others tested. Moreover, the proteins are located on the right part of the sperm. Sperm bind to eggs by means of a spear-like projection called the acrosomal process that forms during activation of the sperm. The bindin is present on the acrosomal projection.

According to Vacquier and his colleagues, bindin has a molecular weight of 30,500. They are now determining the amino acid sequences of the bindins from the two different sea urchin species. The sequences determined thus far are similar but not identical. Presumably the differences would account for the different binding specificities of the proteins.

The other participant needed for the binding of sperm to egg is the sperm receptor on the egg. Several lines of evidence developed by Kenji Aketa of Nagoya University in Japan, William Lennarz of Johns Hopkins University, and Vacquier, among others, indicate that the receptors are glycoproteins (proteins complexed with carbohydrate). For example, if sea urchin eggs are treated before exposure to sperm with an enzyme that breaks down proteins, fertilization is prevented. This result suggests that when the protein is destroyed sperm cannot bind. Moreover, treatment of eggs with a lectin (a plant protein that binds to certain carbohydrates) also prevents fertilization, presumably by binding to the receptors and preventing the sperm from attaching to them.

Aketa, Lennarz, and Vacquier have all isolated glycoproteins from egg membranes that may be the sperm receptor material. The Johns Hopkins workers have shown that when their material is added to the culture medium, it inhibits fertilization by binding to sperm of the same species as those from which it was isolated but it does not inhibit fertilization by sperm of other species. Vacquier and his colleagues have demonstrated that the glycoprotein prepared in their laboratory binds to bindin and that this binding is also species-specific. It is too early to tell whether the materials from the three laboratories are the same, although the molecular weights determined by Vacquier and Aketa are similar.

Much of the research on fertilization has been performed with the sea urchin and other animals whose eggs are fertilized externally. This is largely a matter of convenience in obtaining materials for study. But where mammalian fertilization has been studied the results usually resemble those obtained with sea urchins, although there may be differences in some of the details.

Thus, an obvious implication of the research on sperm binding is that the information acquired might be used to develop new methods of human birth control. The goal would be to devise ways of preventing the binding that would be more reliable than current mechanical techniques for achieving this result. The potential methods would also have the advantage of not disrupting normal sex hormone function in females as contraceptive pills do.

Vacquier, for example, has suggested that one potential birth control method would be the immunization of women against bindin. The idea is that antibodies to bindin would bind with the sperm and prevent them from attaching to the egg. Although the Davis workers have identified bindin on oyster sperm, there is no evidence that mammalian sperm carry it or a similar protein. More work will be required to determine how widespread bindin is in the animal kingdom. Mammalian eggs are already known to carry sperm receptors, however, and an approach to birth control now being investigated in several laboratories is the immunization of females against the sperm-binding components of their eggs (see box on page 1258).

Although a single egg may bind hundreds, or even thousands of sperm (Fig. 1), only one actually fertilizes the egg. Because the sperm and egg each contribute half of the embryo's chromosomes, fertilization by more than one sperm would produce an embryo with too many chromosomes. Such an embryo would be likely to develop abnormally, if at all. Elaborate mechanisms to prevent fertilization by more than one sperm (polyspermy) have evolved.

Sea urchin eggs produce both a fast block to polyspermy, which begins as soon as the first sperm fuses with the egg and lasts for about 60 seconds, and a slow block, which takes over about the time the fast block is fading. Experiments by Larinda Jaffe, who is now at the Marine Biology Laboratory in Woods Hole, indicate that the fast block is the result of changes in the electrical properties of the egg membrane.

One of the properties of cell membranes is that they maintain a voltage difference between the inside and the outside of the cell with the inside being more negative in resting cells. Jaffe found that when cells are fertilized the potential across their membranes increases sharply from a value of −60 millivolts before fertilization to as high as +10 mV immediately afterward. Eggs whose potentials


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climb to 0 mV or higher never become polyspermic whereas eggs whose potentials remain more negative than −10 mV are sometimes fertilized by more than one sperm. Jaffe hypothesized that the positive potentials block the entry of extra sperm, a hypothesis that appears to have been borne out by further experimentation.

When Jaffe deliberately raised the potential of unfertilized eggs to +5 mV by applying an electrical current to them, the eggs were not fertilized even though many sperm could be seen adhering to them. As soon as she turned off the current, fertilization occurred, however. When current was applied to fertilized eggs to lower their potentials to −30 mV, polyspermy resulted. Jaffe, with Meredith Gould-Somero of the University of California at San Diego, has performed a similar series of experiments—with similar results—on the fertilization of the eggs of a large marine worm (*Urechis caupo*). In this case, however, the high potential is maintained for up to 10 minutes after fertilization.

If the fast block to polyspermy is electrical and transitory in nature, the slow block involves major chemical and structural changes in the egg surface and is permanent. Sea urchin eggs are encased in a jelly-like outer covering. Under the jelly is a membrane-like structure called the vitelline layer, which is probably the location of the sperm receptors. The vitelline layer lies immediately over the membrane of the egg itself. And immediately under the egg membrane are a number of small sacs called cortical granules that contain enzymes and other materials. Fusion of the first sperm with the egg membrane (the fertilization step) triggers the cortical reaction during which these granules fuse with the cell membrane and release their contents between it and the vitelline layer. Much of the slow block is the result of the cortical reaction which occurs in the minute or so after fertilization.

One result of this release is the swelling of the vitellin layer which lifts away from the egg. Another result, according to Vacquier and David Epel of the Hopkins Marine Station of Stanford University, is the discharge of an enzyme that destroys the sperm receptors, thus freeing the extra sperm that are already bound and preventing the binding of any more. Vacquier says that the egg glycoprotein that he postulates to be the sperm receptor disappears from the egg right after the cortical granules release their contents. This would be additional evidence that the glycoprotein is actually the receptor.

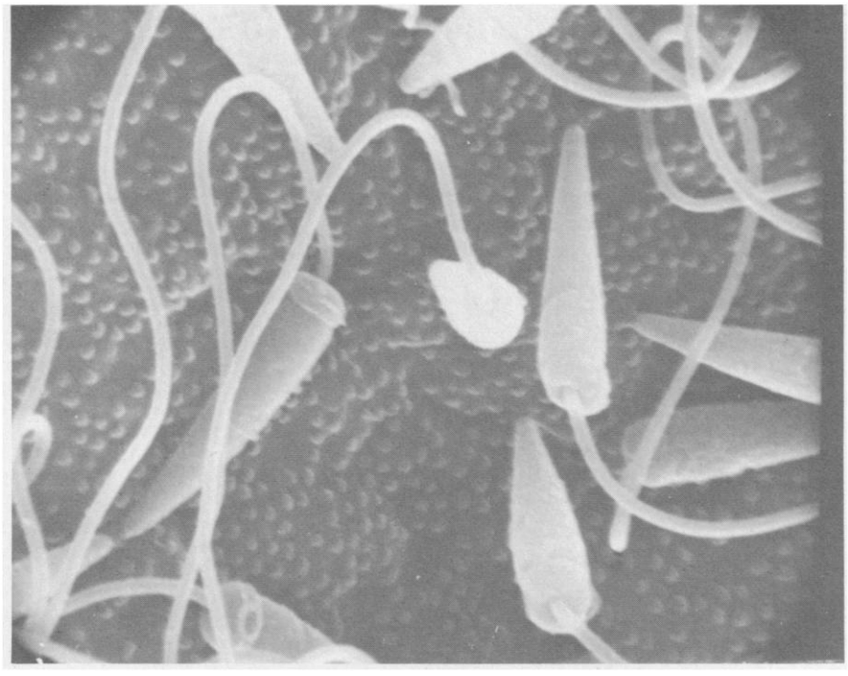

Fig. 1. Scanning electron micrograph of several sperm attached to the vitelline layer of a sea urchin egg (*Strongylocentrotus franciscanus*) (×10,500). [Source: Mia Tegner, Scripps Institution of Oceanography]

A third consequence of the release of the cortical granule contents is the formation of the fertilization membrane from the vitelline layer that has now been lifted off the egg. When the fertilization membrane is first formed, it is soft and easily dissolved by agents such as detergents. But after a few minutes it becomes hard and insoluble and a highly effective barrier to penetration by additional sperm.

According to Bennett Shapiro of the University of Washington, and Glenn Hall, who is now at Davis, the hardening process involves the formation of bonds between the tyrosine residues of the glycoproteins of the fertilization membrane. In effect, the glycoproteins are cross-linked to form one large rigid molecule. Shapiro and Hall, working independently, have identified an enzyme called an ovoperoxidase that is released from the cortical granules. In the presence of hydrogen peroxide the enzyme catalyzes the formation of the cross-links.

Shapiro has also shown that the hydrogen peroxide is produced by the egg at the right time soon after fertilization. Its formation requires oxygen, and Shapiro thinks that this phenomenon may account for the marked increase in oxygen consumption by newly fertilized eggs that was noted by Otto Warburg some 70 years ago but not explained until now.

Moreover, hydrogen peroxide and peroxidase are toxic to several kinds of sperm, including those of mammals. If these agents kill sperm in nature and not just in the test tube, Shapiro says, then their formation may be yet another mechanism for preventing polyspermy.

Investigators have long wondered just what signals trigger the profound changes the egg undergoes as a consequence of fertilization. Before fertilization the egg is relatively quiescent, carrying on just enough activity to maintain itself. Within a few minutes of sperm penetration, however, it not only establishes the fast and slow blocks to polyspermy but also experiences a marked increase in synthetic activity as it gears up for cell division.

Epel points out that the series of events constituting egg activation can be divided into an early phase beginning within 3 seconds of fertilization and lasting for about 1 minute and a later phase that begins about 5 minutes after fertilization. The early phase includes a rapid movement of sodium ions from the culture medium into the egg. Because of Larinda Jaffe's results, the fast block to polyspermy is now thought to be the result of the change in voltage potential of the cell that is caused by this rapid influx of positive ions. The cortical reaction, which constitutes the slow block to polyspermy, is also part of the early phase. The later phase includes the marked increase in protein and nucleic acid synthesis required for cell division.

An observation made in 1937 by Daniel Mazia, now at the University of Cali-

# Contraception: An Antipregnancy Vaccine?

Name a birth control method and you can name a problem with it. Some methods, such as rhythm and contraceptive foams, are not very reliable; others, the condom and diaphragm, for example, are more reliable but are often not used because of the inconvenience; a third class of contraceptives includes the pill and intrauterine devices, methods that are very effective but that may be associated with unpleasant or even dangerous side effects. Because—or perhaps in spite—of these problems, investigators are still searching for the ideal contraceptive technique.

An approach that many think promising, although it is still in the very early stages of research, is the development of a vaccine to prevent pregnancy. Such a vaccine could be directed against any of several targets needed for the initiation or maintenance of pregnancy. Investigators have already tried with some success, at least in experimental animals, to immunize females against sperm or against certain hormones needed for the maintenance of pregnancy. Now they are learning that fertilization can also be blocked in several species of animals by immunizing the females against components of their own eggs.

Early suggestions that this was possible came from the laboratory of C. Alex Shivers at the University of Tennessee. Shivers and his colleagues prepared antibody that specifically binds to the zona pellucida of hamster eggs and forms a precipitate on the zona surface. The zona pellucida, which is an envelope of transparent, spongy material surrounding mammalian eggs, contains the receptors by which sperm attach themselves to the eggs. Shivers hypothesized that the antibodies, by binding to the zona, would block the access of sperm and thus prevent fertilization. The Tennessee workers subsequently showed that antibody treatment does prevent sperm binding to and fertilization of eggs in the test tube.

Meanwhile, several additional investigators* were also turning their attention to the egg as a possible target for immunological fertility control. They, and also Shivers, found that not only do antibodies to the zona block fertilization in the test tube, but that they also completely—but temporarily—prevent conception in several species of living animals, including the hamster, rabbit, mouse, and rat. At present, the researchers do not know whether the antibodies prevent sperm attachment by binding to the sperm receptors themselves or whether they bind to some other zona component and physically block sperm access to the egg.

Most of the investigators have used passive immunization procedures. For passive immunization, the test animals are injected with antibodies directed against the antigen in question. (An antigen is any substance that elicits antibody production.) This means that the antibodies must first be produced in some other animal, either of the same species as the test animals or more often in different species, which have been injected with the antigen.

But active immunization, in which the test animals produce the antibody themselves, is also possible. For example, Ralph Gwatkin has actively immunized female hamsters and rabbits against conception with solubilized preparations of zona material. The animals make antibodies to the zona, and these presumably cause the infertility.

However the immunization is achieved, the animals appear to suffer no ill effects. They do not experience any dangerous allergic reactions that could result from injection with an antigenic material. More research is needed to determine whether repeated injections would trigger such reactions, however. Moreover, the animals eventually regain full fertility, usually after a few to several estrous cycles. Reversibility is one of the requirements to be met if an antipregnancy vaccine is ever to be developed for humans.

A second requirement is that the antibodies produced must be directed only against egg antigens. Antibodies that attack other tissues might cause severe damage to the immunized animal or individual. In earlier work on passive immunization, the investigators frequently used extracts of whole ovary, rather than purified zona material, to elicit the antibody production. They then had to treat the antibodies to remove those directed against tissues other than the egg before the material could be injected into the test animals. In theory, the use of purified zona material to elicit the antibody production might appear to eliminate the need for the treatment to remove unwanted antibodies. But results from the laboratory of Anthony Sacco indicate that this is not the case in practice. In contrast, Gwatkin says that tests indicate that the antibodies produced in response to the zona material he uses for active immunization are specific for zona. The reason for this discrepancy is unclear.

A third requirement for an antipregnancy vaccine is that it must be possible to use an antigen from some species other than the human to elicit antibody production. Human zona material would necessarily be in short supply and its widespread use for immunization would be impractical. Moreover, the evidence indicates that zona material may not be very effective in evoking antibody production in the same species from which it was prepared. Zona antigens prepared from other species might work in humans, however. Sacco, for example, has shown that antibody against pig zona pellucida also reacts with human zona material. Moreover, Shivers now has evidence that antibodies to pig zona block the attachment of human sperm to eggs in culture. Finally, Gwatkin has observed similar phenomena with antibodies against bovine zona material.

Whether the antibodies will do the same in vivo is unknown. Although testing of the immunological contraceptive procedures in nonhuman primates is beginning, several years of animal research will be required to determine whether the methods are safe and reliable enough to warrant testing in humans.

One indication that antibodies might prevent conception in vivo is that Shivers and Sacco have found that some women, who are infertile for unknown reasons, have high concentrations of antibodies to zona pellucida in their bloodstreams. Little or none of the antibody occurs in the blood of men and fertile women. Two of the women that Shivers and Sacco studied eventually had babies, an indication that the condition might be reversible. Thus, although much work remains to be done, investigators are hopeful that they can develop an antipregnancy vaccine for controlling human fertility.—J.L.M.

*They include M. C. Chang and Y. Tsunoda of the Worcester Foundation for Experimental Biology, Ralph Gwatkin of the Merck Institute for Therapeutic Research, Garth Nicolson of the University of California at Irvine, Anthony Sacco, first with Shivers at Tennessee and more recently at Wayne State University School of Medicine, and Ryuzo Yanigimachi of the University of Hawaii School of Medicine.

fornia at Berkeley, suggested that an increase in the concentration of calcium ions in the newly fertilized egg was somehow involved in the post-fertilization changes. Although the suggestion was based on experiments that would be considered crude by today's standards, it nevertheless appears to be right on target. Recent results confirm that calcium ions trigger at least some of the early changes in the fertilized egg, including the cortical reaction.

For example, Richard Steinhardt, of the University of California at Berkeley, and Epel showed that sea urchin eggs can be activated to undergo the changes characteristic of fertilization, without any help from sperm, by exposing them to a calcium ionophore. (An ionophore is a chemical, usually an antibiotic, that can pick up ions and carry them across membranes that would normally be impermeable to the ions.) The ionophore used by Steinhardt and Epel activates eggs of all the species tested.

Although these results strongly suggested the involvement of calcium ions in egg activation they did not actually prove that the calcium ion concentration increases in the egg as a result of fertilization. A direct demonstration of such an increase was achieved, however, by Ellis Ridgway of the Medical College of Virginia, Lionel Jaffe of Purdue University, and their colleagues, who used a luminescence technique to detect calcium ions in the egg.

These investigators first injected eggs of the medaka, a small freshwater fish, with a chemical called aequorin. When aequorin is exposed to calcium ions it gives off light in proportion to the calcium ion concentration. Aequorin in unfertilized eggs was barely luminescent, indicating a very low concentration of free calcium ions. But within 2 seconds of activation by sperm the light output increased as much as 15,000-fold. Jaffe says that in a dark room the flash of light is visible to the naked eye. Steinhardt and his colleagues have since used aequorin to demonstrate a transient increase in calcium ions in sea urchin eggs.

For several reasons, most investigators agree that the source of the increased concentrations is the internal calcium stores of the egg itself. For example, fertilization of sea urchin eggs by sperm can proceed in the absence of calcium ions provided the sperm have already been activated, a process that does require calcium. Moreover, Steinhardt and Epel found that calcium did not have to be present in the fluid surrounding sea urchin eggs for them to be activated by the ionophore. Activation, whether by sperm or by the ionophore, can apparently free calcium that was sequestered in a bound form within the egg.

The natural trigger for this calcium ion release within the egg may be the discharge of small quantities of calcium ion by the fertilizing sperm. Ridgway and Jaffe have recently used image intensification techniques to observe what happens to aequorin-injected eggs when they are fertilized. What they saw was a wave of increased calcium ion concentrations beginning at the point of sperm penetration and terminating at the opposite pole of the egg. The investigators consequently inferred that the fertilizing sperm triggers the wave by releasing a small quantity of calcium ions at the site of fusion. Shapiro has made a similar suggestion on the basis of his observation that sperm accumulate a relatively large quantity of calcium when they are activated, turning them into what he terms "calcium bombs" because of the potential effects of the accumulated calcium on the soon-to-be fertilized egg.

Despite the critical role played by calcium ions in the early stages of egg activation, there is evidence that it is not necessary for the later synthetic phase. The early phase is not a prerequisite for the later one, as has been shown by Epel, Steinhardt, and Mazia. Some treatments, such as incubation in ammonia, will start the synthetic reactions in the absence of the cortical reaction and without significantly increasing the internal calcium ion concentrations of the egg.

### Increased *p*H and Egg Activation

What does appear to turn on the synthetic phase of egg activation is a loss of hydrogen ions from the egg and a consequent increase in its internal *p*H. The fast block to polyspermy occurs as a result of the rapid influx of a small quantity of sodium ions. Somewhat later, at about 90 seconds after fertilization, there is a larger influx of sodium ions into the egg. Epel and his colleagues have shown that, during this larger influx, the sodium ions moving into the egg are exchanged for hydrogen ions moving out. They think that activation is not the result of the sodium ion influx but that it is due to the outward movement of hydrogen ions, which should produce a decrease in the *p*H of the egg cytoplasm, because activation by ammonia can proceed in the absence of sodium. In this case, ammonia penetrates as the unprotonated base and picks up a hydrogen ion in the cell, thus directly raising the *p*H of the cytoplasm. Steinhardt and his colleagues have measured the *p*H in sea urchin eggs activated by sperm or by ammonia. In all cases the *p*H increased as expected.

Consequently, the investigators have concluded that a low intracellular *p*H suppresses the metabolic activities of the unfertilized egg. The rapid loss of hydrogen ions following fertilization then raises the intracellular *p*H and somehow activates the synthetic machinery of the cell. Just how the *p*H change activates the machinery is as yet unclear.

The ion movements evoked in the egg by fertilization closely parallel those in sperm undergoing activation before fertilization. In order for sperm to fertilize eggs they must undergo a series of changes collectively called the acrosomal reaction. These changes are initiated when the sperm come in contact with the jelly layer that surrounds the eggs of sea urchins and related animals. Mammalian sperm also undergo an acrosomal reaction although it is not as dramatic as that observed in sperm of the sea urchin.

During the acrosomal reaction the membrane of the acrosome (a large protein-filled sac sitting on the head of the sperm) fuses with the sperm membrane. The vacuole then turns itself inside out to release its contents to the outside of the sperm. Among these contents are an enzyme that digests the jelly layer and thus helps the sperm to make its way through this layer to the egg and bindin, the protein shown by Vacquier to bind the sperm to specific receptors on the egg.

Calcium ions are needed for the fusion of the acrosome with the sperm membrane just as they are for the fusion of the cortical granules with the egg membrane, according to Epel, Shapiro, and Lewis Tilney of the University of Pennsylvania. Shapiro calculates that the sperm take up enough calcium to make the concentration of the ion in the sperm about three times that in the egg.

Formation of the acrosomal process is another component of the acrosomal reaction. The process is composed of filaments of polymerized actin. (Actin, which is one of the major contractile proteins of muscle, is found in many kinds of cells.) According to Tilney, formation of the process seems to depend on an increase in the intracellular *p*H of the sperm that results from a hydrogen ion exodus in exchange for a sodium ion influx. He hypothesizes that the increased *p*H causes the release of actin from the proteins with which it is otherwise bound and thus allows the actin to polymerize to form the acrosomal filaments.

Thus, the activation of sperm, although seemingly quite different from that of the egg, appears to be accomplished by similar changes in ion movements. Primed for action, the sperm can now fertilize the egg and initiate a new life.—JEAN L. MARX