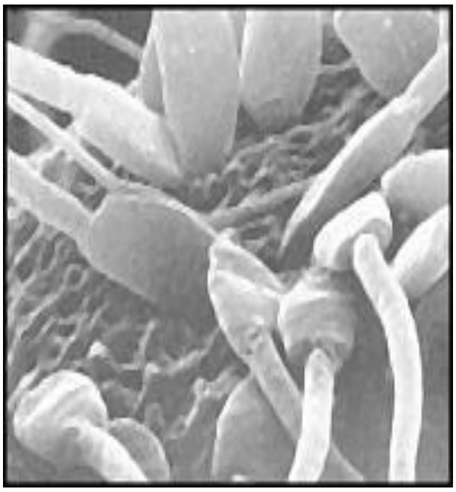
Fertilization



- Fertilization is the union of two haploid gametes to reconstitute a diploid cell - a cell with the potential to become a new individual.
- Fertilization is a series of steps that might be said to begin when egg and sperm first come into contact and end with the intermingling of haploid genomes.
- Prior to fertilization, the two gametes must become fully mature and be transported to a site within the female - the oviduct - that will support their interactions with one another.
- The first of many challenges following fertilization is to become multicellular, and the one-cell embryo rapidly cleaves into 2, 4, 8 and more cells. It then starts to do some interesting things like develop a discrete inside and an outside.
- Finally, the embryos of many species start to secrete hormones that ensure their survival - a process called maternal recognition of pregnancy.

Gamete Transport

Fertilization depends upon the two gametes bumping into one another. In species with internal fertilization, which includes all mammals and birds, both sperm and egg must be transported into the oviduct, which serves as the site of fertilization.

Sperm Transport

Semen is ejaculated and deposited initially into one of three sites:

- The vagina (e.g. humans, cattle, and rabbits)
- The uterus (e.g. horses, rodents)
- The cervix (e.g. pig).

- In species such as dogs, semen is probably deposited largely into the vagina, but also forced into the uterus.
- Despite these differences in deposition site and significant differences in the number of sperm ejacuated, there is remarkably little variation among species in the total number of sperm that reach the oviducts.
- Typicially, a few hundred to a few thousand sperm reach the oviducts following a single mating, which usually represents far less than one percent of the sperm in the ejaculate.

The vagina

Represents a hostile environment for sperm, and their continued survival depends on getting into more hospitable regions of the female tract. In their journey from vagina to oviduct, sperm must overcome a series of barriers, each of which eliminates a substantial proportion of the original population of sperm.

The cervix

Connects the vagina to the uterus. The cervical canal follows an irregular, tortuous route, and the epithelium contains many deep crypts.

The cervical epithelium is richly endowed with mucus-secreting cells, and, as a consequence, the lumen is filled with mucus. Interestingly, the consistency and viscosity of cervical mucus is under endocrine control. When estrogen levels are high and progesterone levels low, as occurs prior to ovulation, cervical mucus becomes watery and its mucin strands assume a parallel orientation. This state apparently greatly facilitates passage of sperm through the cervical canal. Conversely, when progesterone concentrations are high, as in the luteal phase of the cycle, cervical mucus becomes exceptionally viscous and disorganized, which largely precludes entry of sperm into the uterus.

The uterus

Does not present an active barrier, but sperm must somehow be transported directionally along its length.

Studies in several species have shown that sperm are able to get from the distal uterus to the oviducts in times as short as a few minutes, which is much too fast to be explained by sperm motility. Moreover, dead sperm and inanimate sperm-sized particles are rather efficiently transported upward through the uterine lumen.

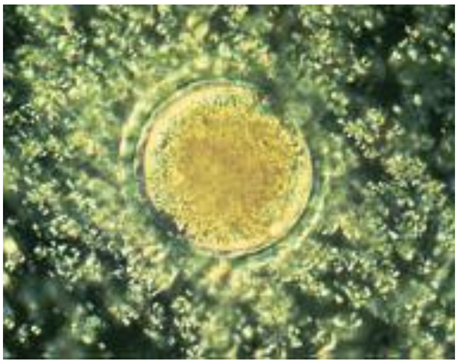
The conclusion from these types of studies is that sperm transport in the uterus is largely a result of uterine contractions, and that sperm motility plays a minor if any role in the process.

In most, but not all species, the uterus is also a site hostile to sperm. In many animals, sperm within the uterus are rapidly phagocytosed. In other cases, sperm can remain viable in the uterus for several days, but their fertility rapidly declines. There are some dramatic exceptions to these general observations.

The uterotubal junction

Is the region joining the tip of the uterine horn to the oviduct. The morphology of this region varies considerably among species, and and this structure appears to be a significant barrier to sperm especially in animals like rodents and pigs where huge numbers of sperm are deposited directly in the lumen of the uterus.

Egg Transport



- Mammalian eggs are ovulated from ovarian follicles as cumulus-oocyte complexes, which consist of the oocyte embedded in a cluster of follicle cells. The image to the right shows such a structure from a cow - the oocyte is encased in its zona pellucida, which is somewhat obscured by a cloud of follicle cells.
- In order to reach the site of fertilization, the ovulated egg must be picked up and transported into oviduct through an opening called the ostium. In most mammals the ovarian end of the oviduct flares into a funnel-shaped structure called the fimbria, which is positioned to partially cover the ovary. The fimbria is densely covered with ciliated epithelial cells, which beat toward the ostium and propel the cumulus-oocyte complex into the oviduct.
- In species such as the rodents and dogs, the ovary is enclosed completely or nearly completely in a thin membrane called the bursa. Because the ostium of the oviduct is inside the bursa, the eggs are essentially trapped after ovulation with no where to go except into the oviduct.
- Once an oocyte enters the oviduct, it is propelled by ciliary motion down into the ampulla, where fertilization takes place. The oviduct provides the appropriate environment not only for fertilization, but for early embryonic development, and it is important that the embryo remain there for a period of about three days.

The Fertilizable Lifespan of Gametes

In most species, both sperm and egg have a short fertilizable lifespan, and once they are delivered into the female tract, the clock starts ticking. What this means, of course, if that mating or insemination must coincide closely with ovulation. If sperm are deposited many days before the egg reaches the oviduct, there is little chance that they will survive to fertilize. Conversely, if sperm reach the oviduct several days after ovulation, they will certainly find an egg that has long since degenerated.

Structure of the Gametes before Fertilization

Fertilization presents some major challenges to both sperm and egg:

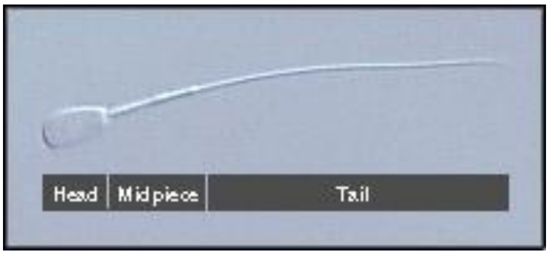
The fertilizing sperm must somehow recognize, bind to and ultimately traverse the zona pellucida surrounding the egg. It then must bind to the plasma membrane of the egg.

The egg must not only respond to the fertilizing sperm in a number of ways, but actively prevent more than one sperm from fertilizing it. Fertilization by more than one sperm is bad.

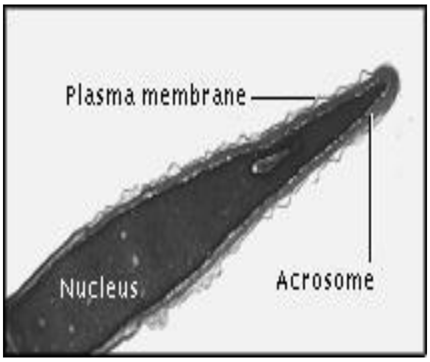
In their mature form, both sperm and egg possess structures that allow them to fulfill these mission objectives.

Structure of the Sperm

Mature sperm, know formally as spermatozoa, have a morphology that most people over the age of ten would recognize immediately. The nucleus is contained within the head, which, for most mammals, has a flattened, oval shape. During spermiogenesis, the haploid sperm cell develops a tail or flagellum, and all of its mitochondria become aligned in a helix around the first part of the tail, forming the midpiece. The entire cell is, of course, enveloped by a plasma membrane.



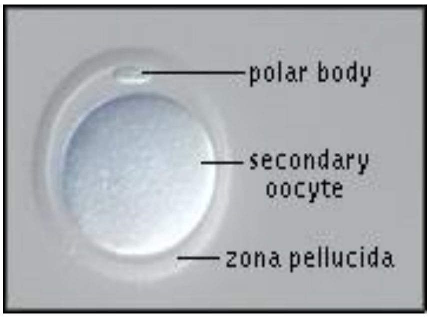
The other structure in the mature sperm that plays a critical role in fertilization is the acrosome. The acrosome is, in essence, a gigantic lysosome that forms around the anterior portion of the nucleus. It is bounded by a membrane that is considered to have two faces - the inner acrosomal membrane faces the nucleus, while the outer acrosomal membrane is in close contact with the plasma membrane.



The image to the right shows the front end of a stallion sperm, viewed with an electron microscope. The ruffled appearance of the plasma membrane is an artifact of fixation. The acrosome is the dark band of material between the plasma membrane and nucleus - inner and outer acrosomal membranes are not clearly visible at this magnification (image courtesy of Carol Moeller)

The function and fate of the acrosome is discussed in the next section on fertilization.

<u>Structure of the Egg</u>



Most mammals ovulate an "egg" that has matured into a secondary oocyte; it is always the secondary oocyte that is fertilized. The secondary oocyte is produced along with the first polar body as a result of the first meiotic division. Both of these cells are encased in a thick glycoprotein shell called the zona pellucida. The image to the right shows a secondary oocyte from a mouse; resic cells have been stripped away.

Genetically, the secondary oocyte that arrives in the oviduct is in metaphase of the second meiotic division. The metaphase plate is located inside the oocyte immediately below the first polar body.

The final structural feature of the egg that serves a critical function during fertilization is a set of cortical granules. During oogenesis, the oocyte develops thousands of small membrane-bound granules that accumulate in the cortical cytoplasm, just beneath the plasma membrane.

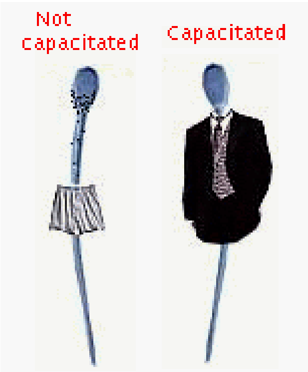
Fertilization

Fertilization is more a chain of events than a single, isolated phenomenon. Indeed, interruption of any step in the chain will almost certainly cause fertilization failure. The chain begins with a group of changes affecting the sperm, which prepares them for the task ahead.

Successful fertilization requires not only that a sperm and egg fuse, but that not more than one sperm fuses with the egg. Fertilization by more than one sperm - polyspermy - almost inevitably leads to early embryonic death. At the end of the chain are links that have evolved to efficiently prevent polyspermy.

In overview, fertilization can be described as the following steps:

1. ***Sperm Capacitation***



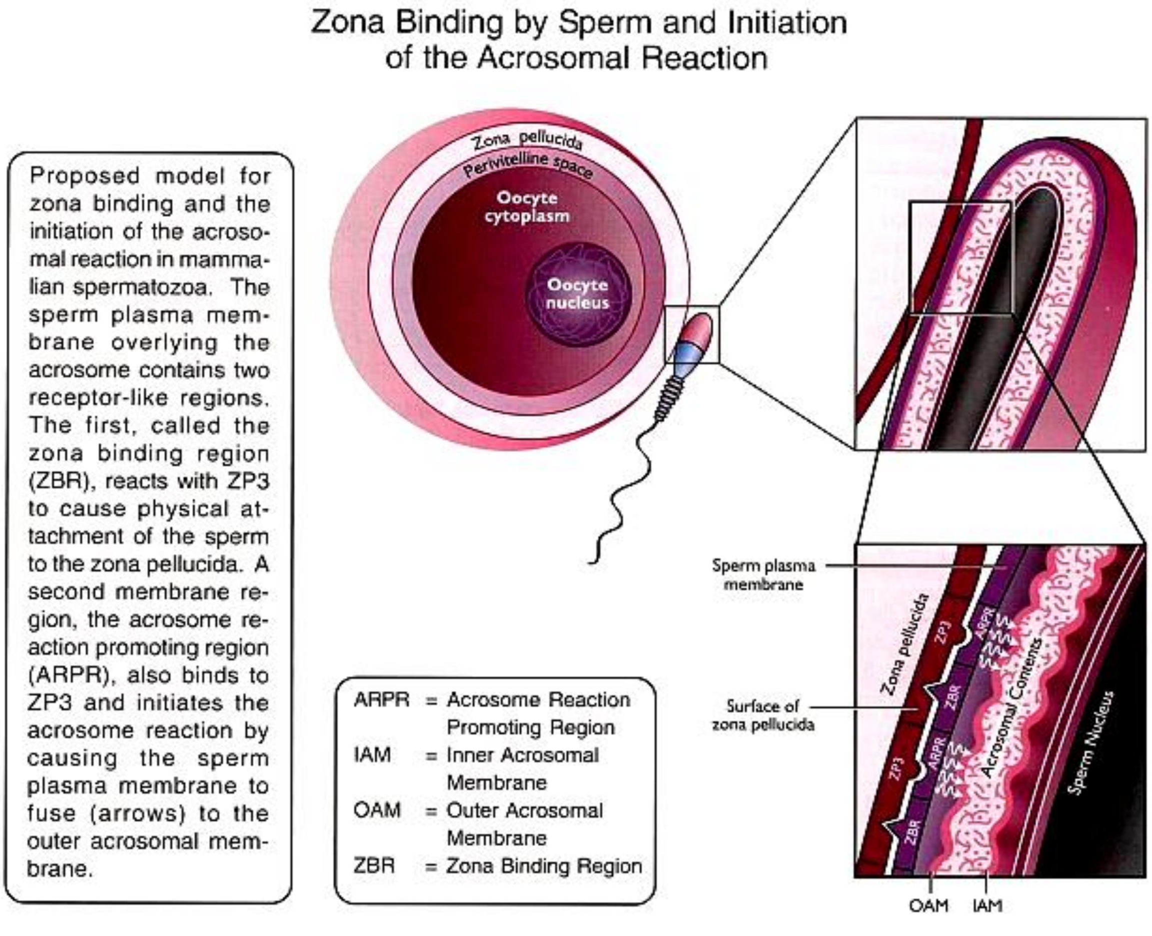
- Freshly ejaculated sperm are unable or poorly able to fertilize. Rather, they must first undergo a series of changes known collectively as capacitation. Capacitation is associated with removal of adherent seminal plasma proteins, reorganization of plasma membrane lipids and proteins.
- Capacitation occurs while sperm reside in the female reproductive tract for a period of time, as they normally do during gamete transport. The length of time required varies with species, but usually requires several hours. The

sperm of many mammals, including humans, can also be capacitated by incubation in certain fertilization media.

- Sperm that have undergone capacitation are said to become hyperactiviated, and among other things, display hyperactivated motility. Most importantly however, capacitation appears to destabilize the sperm's membrane to prepare it for the acrosome reaction, as described below.

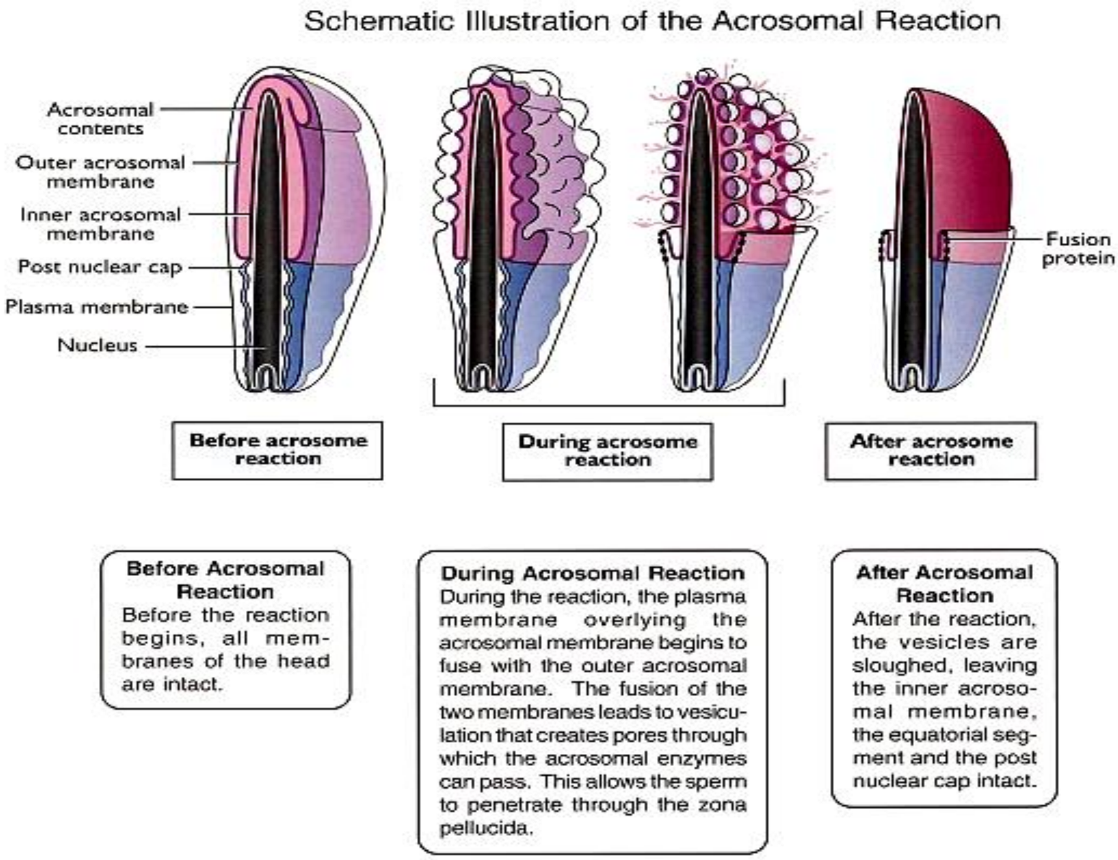
2. Sperm-Zona Pellucida Binding

- Binding of sperm to the zona pellucida is a receptor-ligand interaction with a high degree of species specificity. The carbohydrate groups on the zona pellucida glycoproteins function as sperm receptors. The sperm molecule that binds this receptor is not known with certainty, and indeed, there may be several proteins that can serve this function.



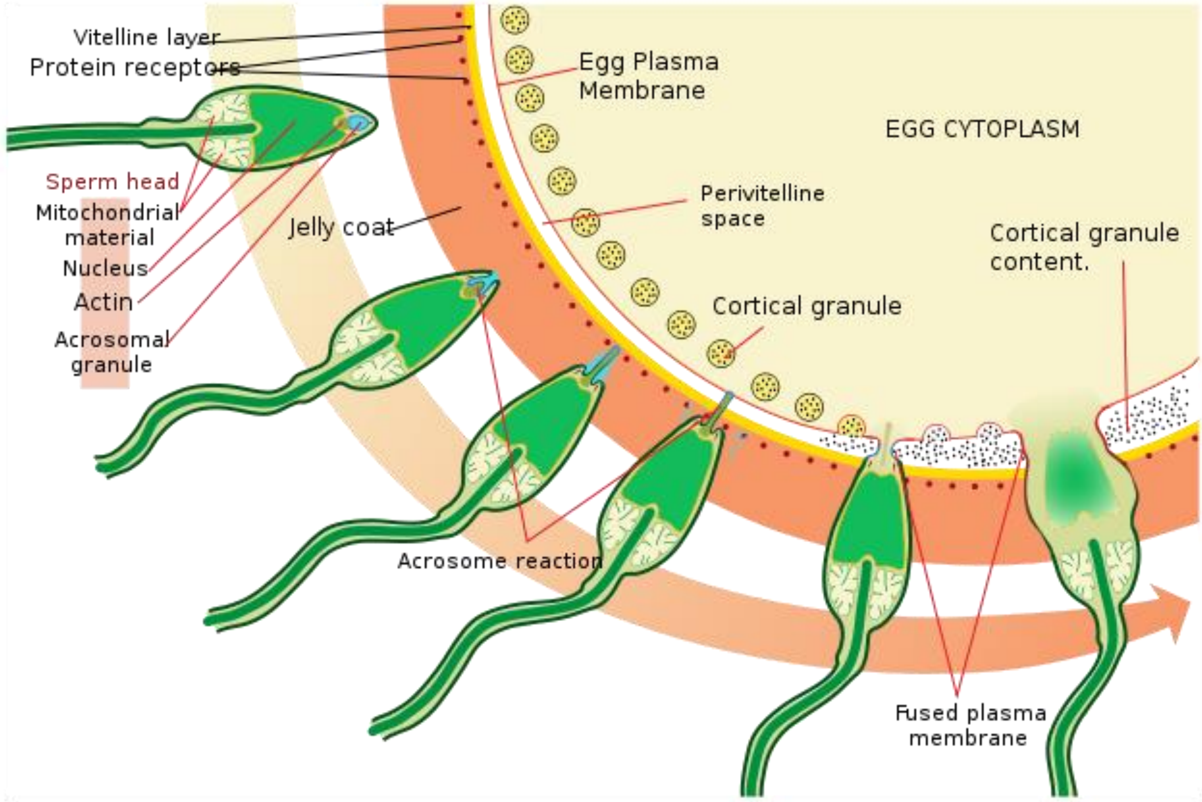
3. The Acrosome Reaction

- Binding of sperm to the zona pellucida is the easy part of fertilization. The sperm then faces the daunting task of penetrating the zona pellucida to get to the oocyte. Evolution's response to this challenge is the acrosome - a huge modified lysosome that is packed with zona-digesting enzymes and located around the anterior part of the sperm's head - just where it is needed.
- The acrosome reaction provides the sperm with an enzymatic drill to get throught the zona pellucida. The same zona pellucida protein that serves as a sperm receptor also stimulates a series of events that lead to many areas of fusion between the plasma membrane and outer acrosomal membrane. Membrane fusion (actually an exocytosis) and vesiculation expose the acrosomal contents, leading to leakage of acrosomal enzymes from the sperm's head.
- As the acrosome reaction progresses and the sperm passes through the zona pellucida, more and more of the plasma membrane and acrosomal contents are lost. By the time the sperm traverses the zona pellucida, the entire anterior surface of its head, down to the inner acrosomal membrane, is denuded. The animation to the right depicts the acrosome reaction, with acrosomal enzymes colored red. Sperm that lose their acrosomes before encountering the oocyte are unable to bind to the zona pellucida and thereby unable to fertilize. Assessment of acrosomal integrity of ejaculated sperm is commonly used in semen analysis.



4. ***Penetration of the Zona Pellucida***

The constant propulsive force from the sperm's flagellating tail, in combination with acrosomal enzymes, allow the sperm to create a tract through the zona pellucida. These two factors - motility and zona-digesting enzymes- allow the sperm to traverse the zona pellucida. Some investigators believe that sperm motility is of overriding importance to zona penetration, allowing the knife-shaped mammalian sperm to basically cut its way through the zona pellucida.



5. ***Sperm-Oocyte Binding***

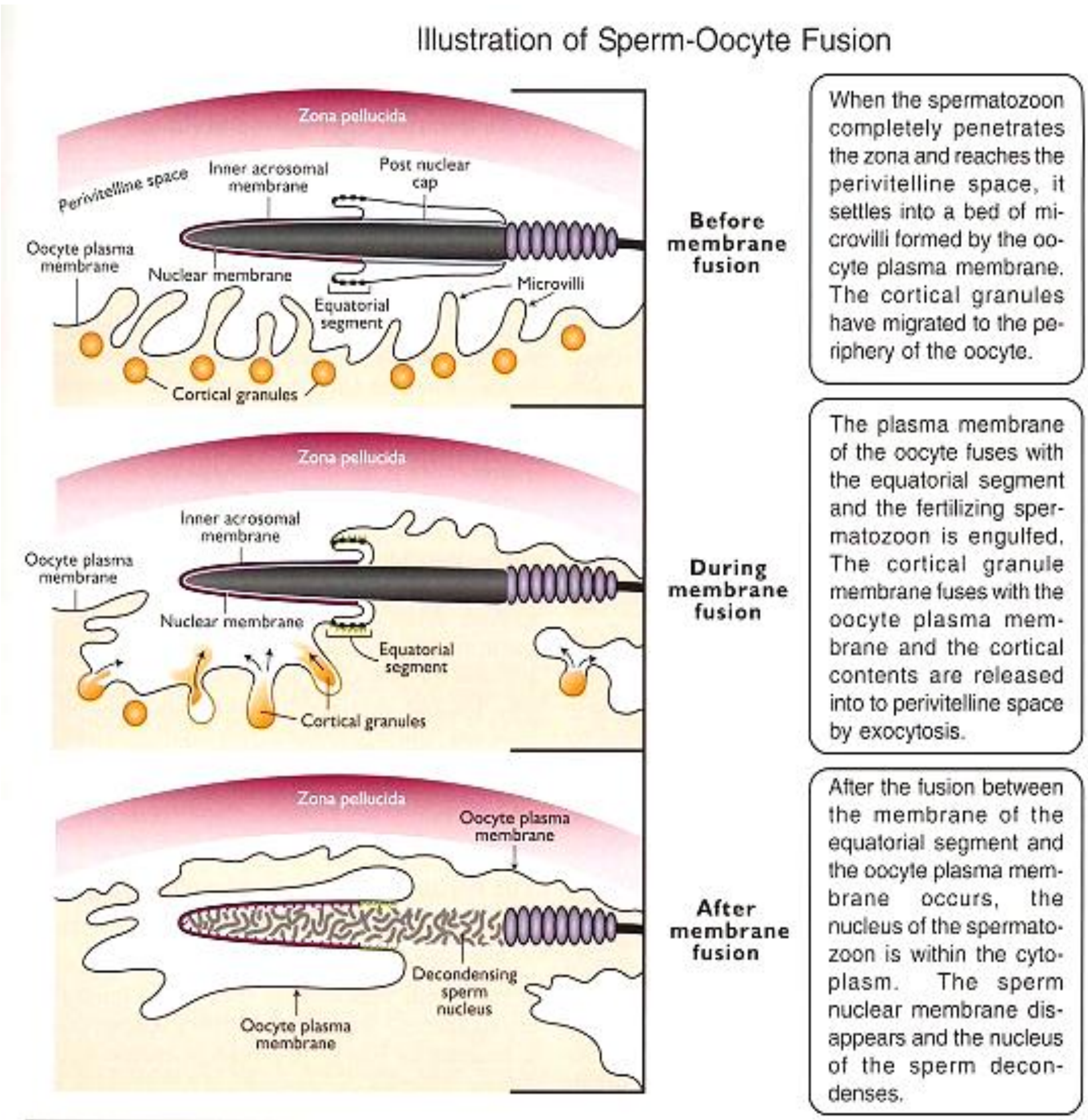
Once a sperm penetrates the zona pellucida, it binds to and fuses with the plasma membrane of the oocyte. Binding occurs at the posterior (post-acrosomal) region of the sperm head.

The molecular nature of sperm-oocyte binding is not completely resolved. A leading candidate in some species is a dimeric sperm glycoprotein called fertilin, which binds to a protein in the oocyte plasma membrane and may also induce fusion. Interestingly, humans and apes have inactivating mutations in the gene encoding one of the subunits of fertilin, suggesting that they use a different molecule to bind oocytes.

6. Egg Activation and the Cortical Reaction

Prior to fertilization, the egg is in a quiescent state, arrested in metaphase of the second meiotic division. Upon binding of a sperm, the egg rapidly undergoes a number of metabolic and physical changes that collectively are called egg activation. Prominent effects include a rise in the intracellular concentration of calcium, completion of the second meiotic division and the so-called cortical reaction.

The cortical reaction refers to a massive exocytosis of cortical granules seen shortly after sperm-oocyte fusion. Cortical granules contain a mixture of enzymes, including several proteases, which diffuse into the zona pellucida following exocytosis from the egg. These proteases alter the structure of the zona pellucida, inducing what is known as the zona reaction. Components of cortical granules may also interact with the oocyte plasma membrane.



7. *The Zona Reaction*

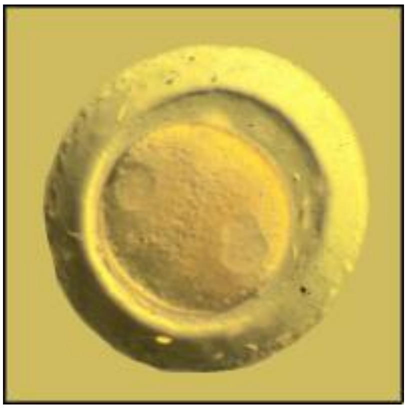
The zona reaction refers to an alteration in the structure of the zona pellucida catalyzed by proteases from cortical granules. The critical importance of the zona reaction is that it represents the major block to polyspermy in most mammals. This effect is the result of two measurable changes induced in the zona pellucida:

The zona pellucida hardens. Crudely put, this is analogous to the setting of concrete. Runner-up sperm that have not finished traversing the zona pellucida by the time the hardening occurs are stopped in their tracks.

Sperm receptors in the zona pellucida are destroyed. Therefore, any sperm that have not yet bound to the zona pellucida will no longer be able to bind, let alone fertilize the egg.

The loss of sperm receptors can be demonstrated by mixing sperm with both unfertilized oocytes (which have not yet undergone the zona reaction) and two-cell embryos (which have previously undergone cortical and zona reactions). In this experiment, sperm attach avidly to the zona pellucida of oocytes, but fail to bind to the two-cell embryos.

8. *Post-fertilization Events*



Following fusion of the fertilizing sperm with the oocyte, the sperm head is incorporated into the egg cytoplasm. The nuclear envelope of the sperm disperses, and the chromatin rapidly loosens from its tightly packed state in a process called decondensation. In vertebrates, other sperm components, including mitochondria, are degraded rather than incorporated into the embryo.

Chromatin from both the sperm and egg are soon encapsulated in a nuclear membrane, forming pronuclei. The image to the right shows a one-cell rabbit embryo shortly after fertilization - this embryo was fertilized by two sperm, leading to formation of three pronuclei, and would likely die within a few days. Pass your mouse cursor over the image to identify pronuclei.

Each pronucleus contains a haploid genome. They migrate together, their membranes break down, and the two genomes condense into chromosomes, thereby reconstituting a diploid organism.

<u>Abnormalities of fertilization:</u>

1. ***<u>Polyspermia:</u>***

Two or more spermatozoa succeeded to penetrate and entire vitelline and participate in fertilization.

2. ***<u>Androgenesis:</u>***

Failure in formation of pronuclei of female, or first polar body.

3. ***<u>Gynogenesis:</u>***

Failure in formation of pronuclei of male, or second polar body.