FERTILIZATION

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12.5 FERTILIZATION

In the earlier sections, you have learnt about the formation and differentiation of the male and female gametes. In this section, we shall describe the process by which the sperm and the egg unite to form a zygote. This process is known as fertilization. Fertilization results in (i) combination of genes derived from 2 parents (fusion of nucleii or amphimixis) resulting in restoration of the diploid chromosomal number in the nucleus, and (ii) generation of a new organism by activating the developmental process.

In this section we will describe the fertilization of sea urchins in which most studies have been done, and the fertilization in mammals which poses several practical problems of study. Prior to the actual fusion of the sperm and the egg nuclei, the germ cells prepare themselves for the fertilization process. In the first subsection, we shall describe such preparatory events. Then you will learn about the fusion process and finally there will be a discussion about the various events that occur subsequent to the zygote nucleus formation.

12.5.1 Event Prior to the Sperm-Egg Fusion

In a number of organisms that have been studied, the sperm remains immotile in the testis or in the semen. The sperm become active and motile when they are close to the eggs. Several factors have been attributed for the activation of the sperm, which include pH, oxygen tension, presence of certain ions etc. Detailed studies in sea urchin have shown that sperm motility is activated only after the sperm have been released in the seawater that changes the pH (Fig. 12.17). In mammals, the definite cause of activation is not known and it is presumed that sperm motility begins with the ejaculation of the semen into the female reproductive tract.

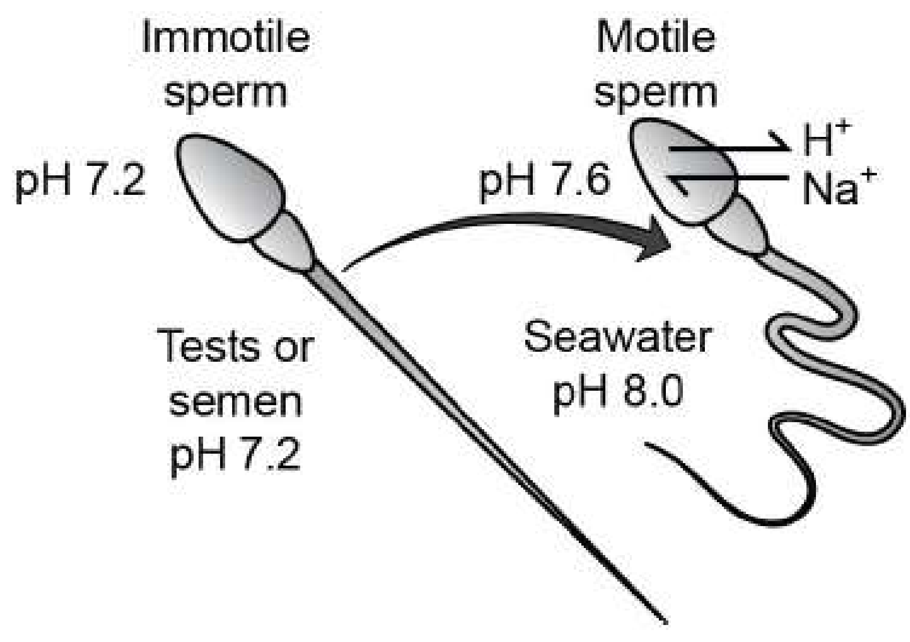

Fig. 12.17: The role of pH in the activation of sperm motility in sea urchins.

12.5.2 Basic Events of Fertilization

The events of fertilization vary from species to species however, it generally consists of 4 major events which are similar both in external and internal fertilization and are as follows:

- Contact and recognition between sperm and egg which ensures that both the gametes are of the same species.
- Regulation of sperm entry into the egg. This is allows generally for only one sperm to enter the egg and inhibits the others.
- Fusion of genetic material of sperm and egg.
- Activation of egg metabolism to start development.

Contact and Recognition Between Egg and Sperm

A major problem in sexual reproduction is to ensure that the spermatozoa and ovum come close to each other. This is achieved in different vertebrate animals by different means. It can be accomplished by the secretion of certain chemicals and mobility of the sperm.

Activation of the sperm does not ensure that the sperm will meet the egg. In instances where fertilization occurs outside the organism, as in external fertilization, chemotactic mechanisms have been evolved to attract the sperm towards the egg. The chemotactic molecules differ even between closely related species. Species-specific sperm attraction has been documented in several species. In fish eggs the substances found near the micropyle attract the sperm. The substances found near the micropyle make the sperm move rapidly to the vicinity of the egg.

This direction for swimming is provided by small chemotactic peptides known as sperm-activation peptides. One such peptide is **resact** (sperm respiratory activating peptide) that has been isolated from the egg jelly of the Atlantic purple sea urchins (*Arbacia punctulata)*. It has been shown that injection of a small quantity of resact causes randomly distributed sperms to change their swimming behaviour and accumulate near the site of injection within seconds. Binding of the sperm membrane to the resact causes the influx of calcium ions from the sea water into the tail and activates cGMP. Increase in cGMP and calcium ions activates the mitochondrial ATP generating apparatus and dynein ATPase which stimulate flagellar movement in the sperms that swim towards the egg. Thus, resact works both as a sperm activating and sperm attracting peptide.

Acrosome reaction

Contact and **interaction** between the sperm and egg jelly of the sea urchin results in the acrosome reaction (Fig. 12.18). The acrosomal vesicle fuses with the sperm cell membrane and releases, by exocytosis its contents consisting of proteolytic enzymes like chymotrypsin, **acrosomin** and proteosomes. The released contents of the acrosomal vesicle digest the jelly coat present on the egg surface and make a path through it, to the vitelline envelope of the egg. The second part of the acrosome reaction involves the extension of the acrosomal process. The inner membrane of acrosome protrudes out as the acrosomal process. The influx of Ca^{2+} which is essential for this process, is mediated through protein **RhoB** present in the acrosomal and mid - piece regions of the sea urchin sperm. The sperm's contact with the egg's jelly coat provides the first set of species-specific recognition events. Another critical species-specific process is the binding event that must occur after the sperm penetrates the jelly and the acrosomal process makes contact with the surface of the egg. This recognition of the extracellular coat is mediated through protein **bindin**, which is again species specific.

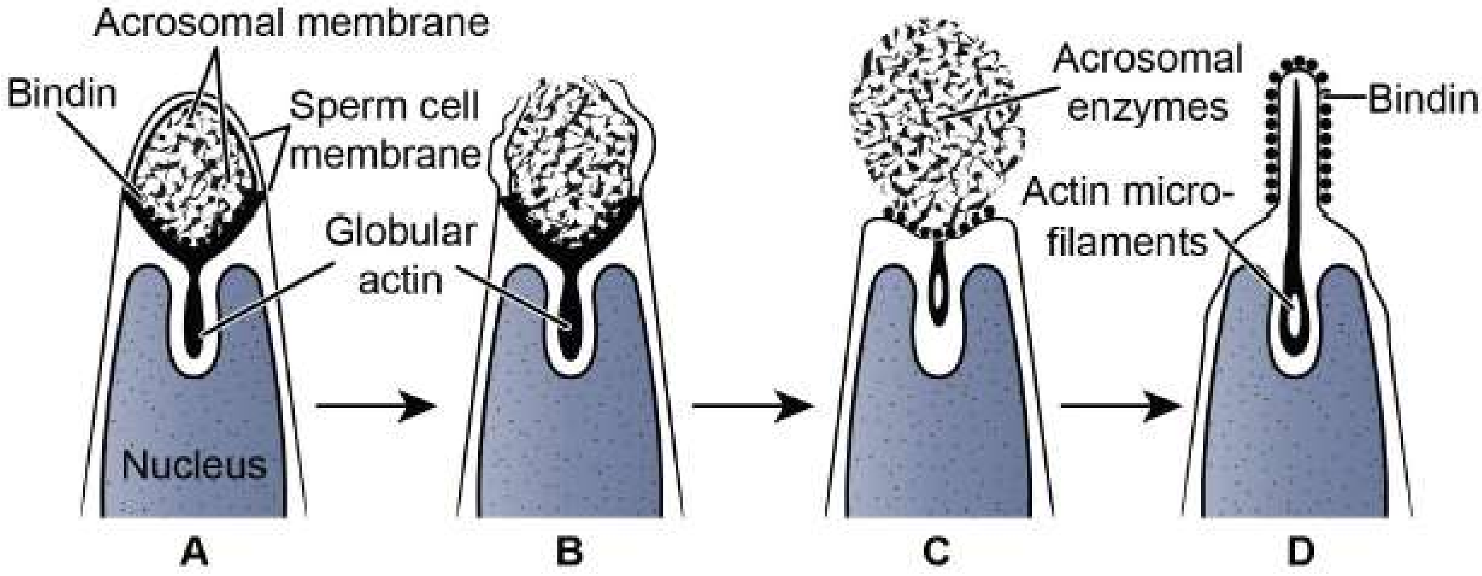

Fig.12.18: Events in the acrosome reaction in sea urchin sperm. A) A sperm head before the beginning of acrosome reaction. The acrosome is intact and is surrounded by the plasma membrane of the sperm; (B&C) At the beginning of the acrosome reaction, there is partial fusion of plasma membrane with the acrosome membrane. Also the acrosomal contents are released and this includes the protein bindin; D) Later stage of acrosome reaction in which acrosome has disappeared and the acrosomal process has extended further, exposing the bindin at the base. The assemblage of actin molecule to produce the microfilaments also occurs at this stage

In sea urchin all regions of the egg cell membrane are capable of fusing with sperm while in other species only certain regions can do so. After the acrosome reaction, the fusion of the egg and sperm membrane causes the polymerization of the actin in egg to form a **fertilization cone** (Fig.12.19). Actin from both the sperm and egg forms a cytoplasmic bridge between them and the sperm nucleus and tail passes through the bridge into the egg. In sea urchin bindin plays a second role as a fusiogenic protein. In sea urchins, the accumulation of Ca^{2+} ions in the sperm appears to control the fusion of acrosomal and sperm membranes. Drugs which inhibit the Ca^{2+} ion movement into the cell are seen to prevent the occurrence of acrosome-plasma membrane fusion.

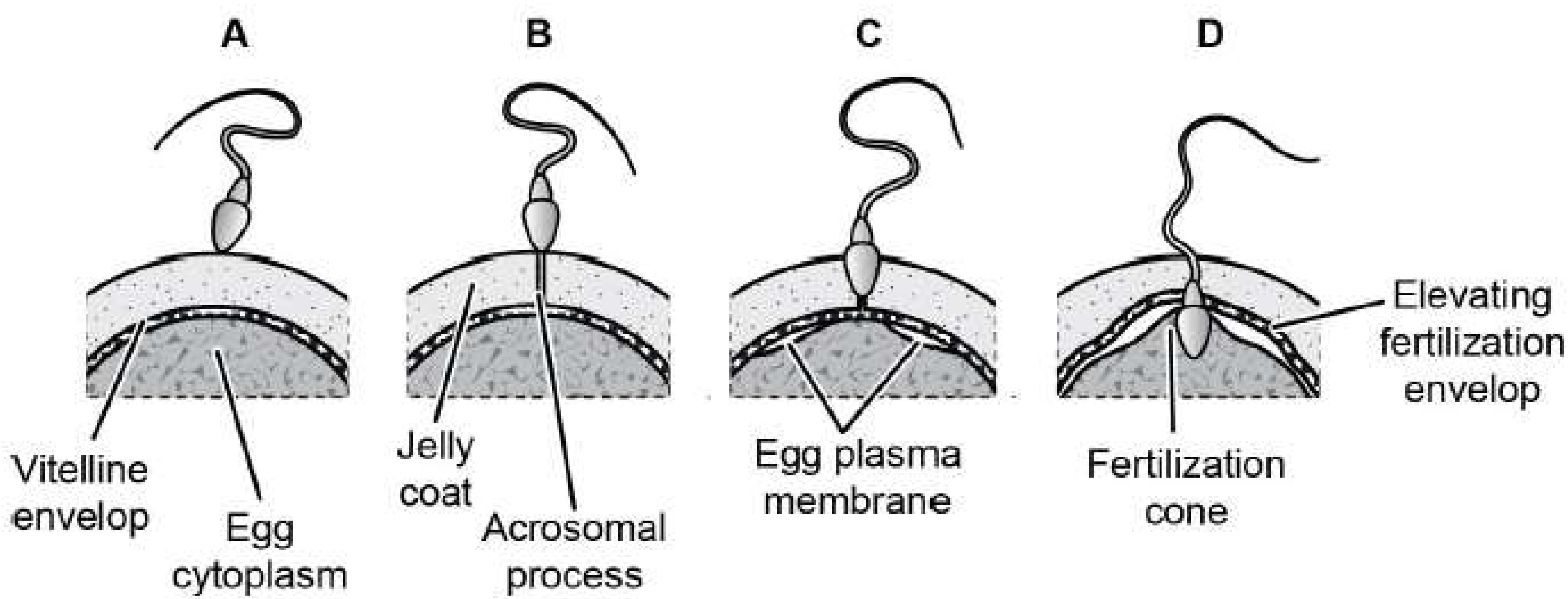

Fig.12.19: Events that occur during the sperm-egg fusion in sea urchin.

12.5.3 Regulation of Sperm Entry in the Egg

When the sperm enters the egg it is important to ensure that only one sperm is allowed to fuse with the egg so that the diploid (n+n =2n) number is maintained. To prevent polyspermy (union of more than one sperm with an ova), different mechanisms have evolved in animals. Two such responses of eggs to prevent entry of more than one sperm are seen in sea urchins: (i) the early response and (ii) the late response. Thus, prevention of polyspermy occurs in two phases: (1). the fast block phase and (2). the slow block phase.

Fast Block Phase

In sea urchins, the fertilization potential causes a temporary change in the voltage across the plasma membrane of the egg from negative to positive potential–from -70 to +20mV. Such potential changes prevent the fusion of more than one sperm with the egg. Recent studies have shown that the potential change is brought about by insertion of a positively charged fusion protein by the first sperm when it fuses with the egg. This fusion protein promotes sperm-egg fusion and the fertilization potential. Once the egg membrane becomes positively charged, insertion of another positive fusion protein by another sperm is not possible, as like charges repel.

Slow Block Phase

The slow block to polyspermy is achieved by cortical reaction, which is a slower mechanical block. The slow block is necessitated by the fact that the fertilization potential is only a temporary phenomenon and the potential of the egg membrane soon returns to negative value. Therefore, at time of fertilization the permanent prevention of entry of additional sperms is necessary.

The cortical reaction begins at the point of entry of the sperm into the egg (Fig. 12.20). The entry of the sperm triggers a wave of exocytosis of cortical granules which spread around the surface of the egg in one minute and fuse with the egg membrane and release their contents into the space between the vitelline envelope and the cell membrane. The perivitelline space is filled with hydrated proteins and mucopolysaccharides which along with the vitelline envelope form the **fertilization membrane**. The fertilization membrane acts in three ways: (i) the membrane increase the distance between the extra sperms attached to its outer surface and the egg plasma membrane through absorption of water; (ii)The peroxides and the peroxidases, released by the cortical granules harden the fertilization membrane and make it resistant to sperm proteases and (iii) the trypsin-like proteases of cortical granules the "cortical granule serine protease" clip off the Bindin receptors and destroy the glycoprotein receptors of the vitelline membrane thereby, dislodging the extra sperms.

Several experiments have demonstrated that Ca^{2+} ions are directly responsible for the cortical granule reaction. This influx of $Ca2^{+}$is not from outside but from the egg cell.

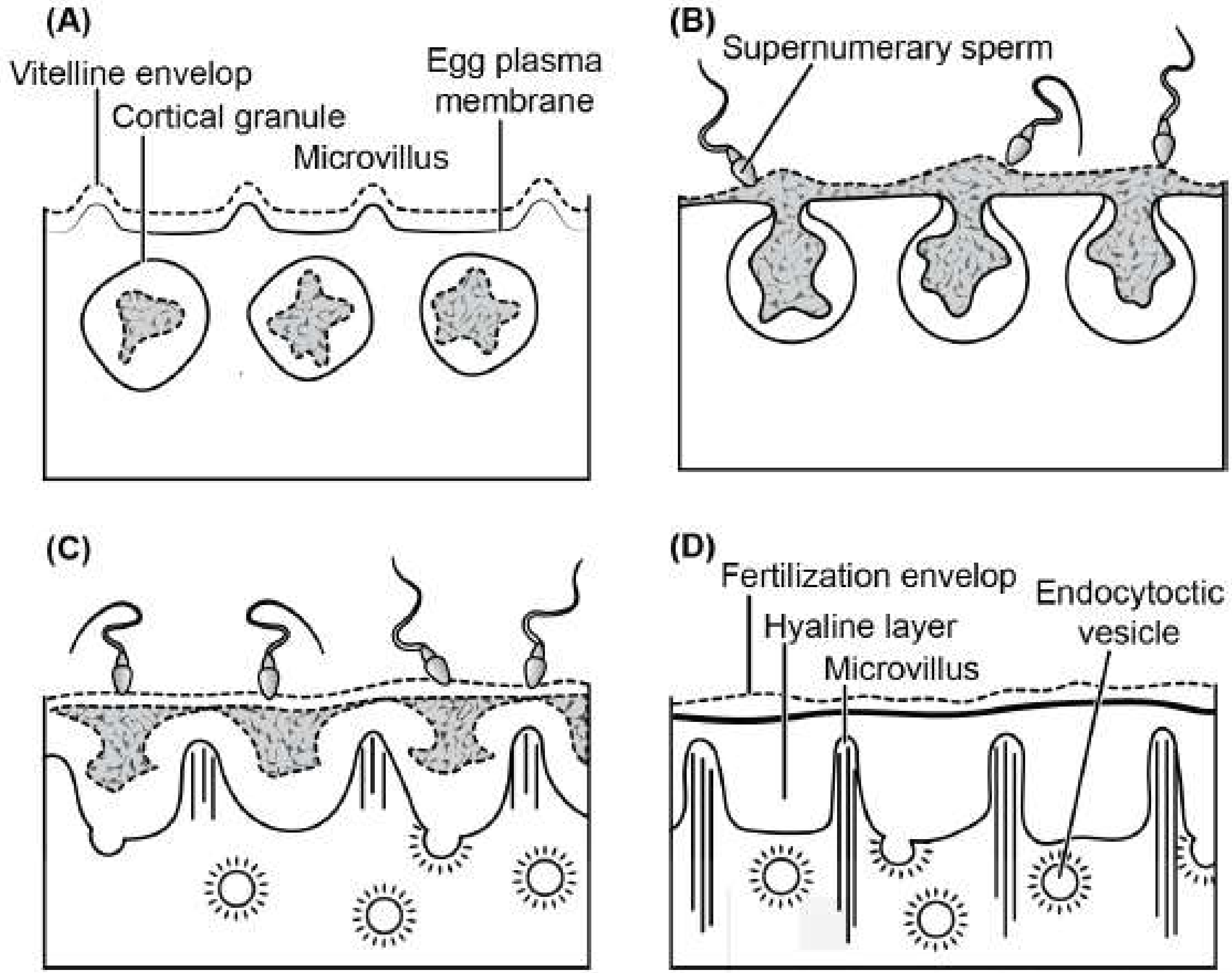

Fig.12.20: Diagram showing cortical reaction and fertilization membrane formation in sea urchin: A) Unfertilized egg with cortical granules, vitelline membrane and plasma membrane with microvilli; B) Just fertilized egg showing exocytosis of cortical granules and beginning of formation of fertilization membrane; C) Elevation of fertilization membrane and completion of cortical granule exocytosis; and D) Fertilized zygote showing elevated fertilization membrane, fully elongated microvilli and hyaline layer.

III. Fusion of Genetic Material

In sea urchins, the completion of second meiotic division in the ovum occurs after the entry of the sperm into the egg. The haploid egg nucleus after the entry of the sperm is known as the **female pronucleus.**

The male pronucleus is also formed in the egg by the breakdown of the sperm nuclear envelope, decondensation of chromatin and the formation of the pronuclear envelope. After the sperm enters the egg, the male pronucleus, separates from the mitochondria and tail and rotates 180º so that the sperm centriole now lies between the sperm and the egg (Fig12.21).

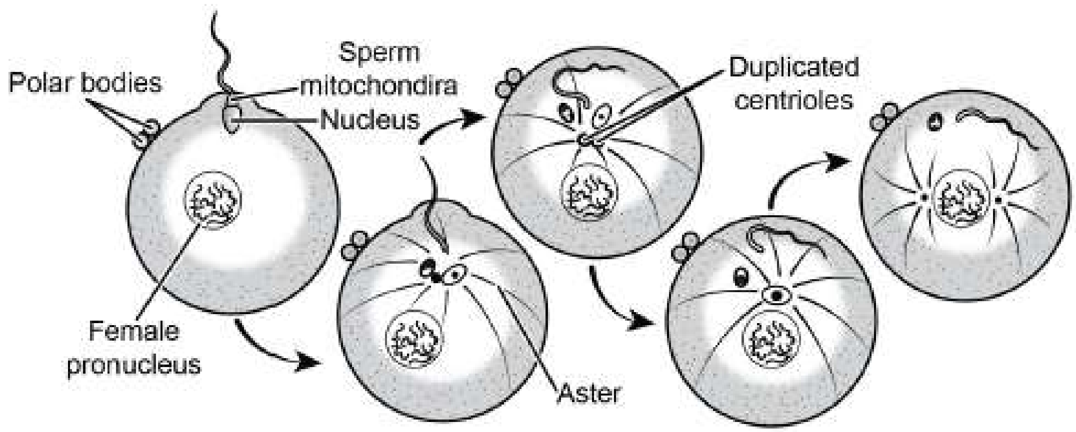

Fig.12.21: Diagrams showing the movement of pronuclei during fertilization in sea urchin.

The centriole brought in by the sperm now acts as a microtubule organizing centre to form the aster by the integration of the microtubules of both egg and the sperm. The two pronucleii migrate towards each other along these microtubules and then fuse to form the diploid zygote nucleus or synkaryon. Usually the nuclear fusion occurs between 10 to 20 minutes after attachment of the sperm to the egg. The mitochondria of the sperm ultimately disintegrate in the cytoplasm.

12.5.4 Fertilization in Mammals

Scientists have found it very problematic to study fertilization in mammals as it is difficult to mimic the conditions that occur inside the oviduct and furthermore, the ejaculated sperm population is highly heterogeneous, containing spermatozoa at different stages of maturation. In addition to this, the female oviduct or reproductive tract is not a passive passage for sperms but is a highly dynamic structure which regulates the transport and maturity of both ovum and sperms. Therefore the sperms undergo two unique processes before they make actual contact with matrix of the egg which contains the **cumulus oophorus.**

The first process is **translocation** which refers to the transport of sperms from vagina to the site of fertilization which is the ampullary region of the oviduct. The transport of sperms is brought about by uterine muscle contractions and to some extent by sperm flagellar motility.

The other process is **capacitation,** in which it is essential for the newly ejaculated sperms to undergo maturation in the female reproductive tract so that they become competent to fertilize the ovum. In mouse, the capacitation period is one hour and in humans 5 to 6 hours. Capacitation mechanism in mammals is generally poorly understood. Usually capacitated sperms consume more oxygen and this is believed to assist the mammalian sperm in penetrating through the surface coats, which protect the eggs and are also responsible for specific sperm-egg interaction.

The processes of both translocation and capacitation are influenced by different regionally specific factors such as Ca^{2+} channel, hyaluronidase enzyme, thermal gradient and chemotactic compounds such as the hormone progesterone.

Acrosome reaction

In mammals, the acrosome reaction commences when the sperms approach the zona pellucida of the egg. This reaction is characterized by the fusion of the outer membrane of the acrosome of the mammalian sperm with the plasma membrane of the egg. After the fusion, the acrosomal membrane vesiculates and releases the acrosomal contents which include several enzymes including **hyaluronidase.** Subsequently, the outer portion of the acrosomal membrane disappears and only the inner portion adjacent to the nucleus remains intact (Fig.12.22). Hyaluronidase acts on the cell surface polysaccharide hyaluronic acid and the sperm plasma membrane protein, PH20 acts as an enzyme and digests the hyaluronic acid.In addition a corona penetrating enzyme, **proteosomes** is also released. The function of these enzymes is to loosen the corona radiata cells and help the sperm to reach zona pellucida. It is believed that the sperm using a protease called **acrosin** present on the sperm head penetrates its way through the zona pellucida. Zona pellucida is believed to possess species specific sperm receptors that prevent interspecific fertilization in mammals. For example, in mouse there are three glycoproteins in the zona pellucida termed ZP_1, ZP_2, and ZP_3, of which ZP_3 is the sperm receptor.

The acrosome reaction in mammals is initiated by an influx of Ca^{2+} into the sperm. In the glycoprotein ZP_3, the sugar moiety acts as a sperm receptor and the protein portion is believed to initiate the acrosome reaction.

Fusion of Sperm and the Egg

In mammals the fusion begins at the equatorial region of the sperm head. The plasma membranes of the two cells become continuous to form a cytoplasmic bridge through which the sperm nucleus enters the egg cytoplasm. Usually the entire sperm including the nucleus, centrioles, mitochondria, plasma membrane and even the flagellar axoneme enters the egg cytoplasm. Once the sperm enters the egg, the fertilization cone forms. The fertilization cone is an extension of the egg cytoplasm which is around the head of the entering sperm. Microfilaments in the fertilization cone virtually draw the sperm into the egg. Inhibitors of microfilament formation such as cytochalasin B inhibit the formation of fertilization cone and sperm entry into the egg. **Zonadhesin** a protein from the acrosome is believed to fix the sperm to the point of attachment and so provides a pivot point for the sperm to enter the zona pellucida.

Prevention of Polyspermy

In mammals fast block to polyspermy has not yet been detected, however a slow block occurs by enzymes from the cortical granules, which modify the zona pellucida proteins (sperm receptors), such that they can no longer bind to the sperm.

The cortical granules contain hydrolytic enzymes which are released into the perivitelline space during cortical reaction. This hardens the zona pellucida which becomes refractive to penetration by extra sperms. The changes in the zona pellucida are called **zona reaction**.

Fusion of egg and sperm pro-nucleii

The mammalian sperm enters almost tangentially to the surface of the egg and the glutathione in the egg cytoplasm allows the uncoiling of sperm chromatin. In mammals, the sperm enters the oocyte in which the nucleus is "arrested" in metaphase of its second meiotic division. The calcium oscillations brought about by the sperm entry inactivates MAP kinase and allows DNA synthesis. The centriole of male pronucleus produces asters, joining the two pronucleii of he sperm and egg and enabling them to migrate towards each other. They become apposed to each other but do not fuse. They remain adjacent to each other until the meiotic divisions, then their nuclear envelopes break down and the paternal and maternal chromosomes mix at a common metaphase plate. Thus a true diploid nucleus in mammals is first seen not in the zygote but at the two-cell stage.

Egg activation

Egg activation requires transient rise in Ca^{+} ions. This rise in calcium seems universal in the fertilization process in the animal kingdom. This rise in calcium is responsible for exocytosis of cortical granules, completion of 2^{nd} meiotic division, resumption of DNA synthesis and general metabolic activation. The 2^{nd} meiotic division results in expulsion of another polar body

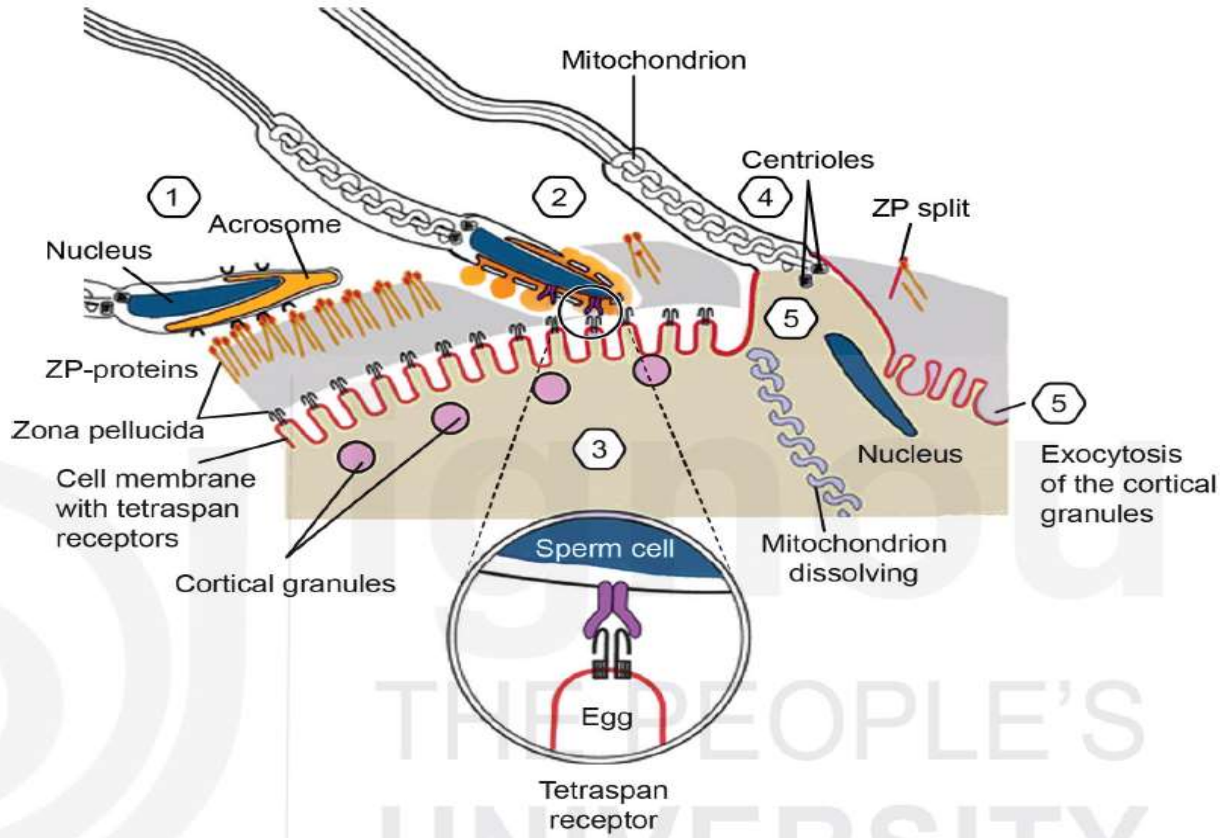

Fig.12.22: Fertilization in a mammal: (1) The sperm head docks with its equatorial zone onto the ZPprotein complex of egg envelope (zona pellucida). (2) Upon contact the acrosomal vesicle is fenestrated and releases a collection of hydrolytic enzymes which by their action form a slit into which the rotating spermatozoan moves, propelled by the flagellum. (3) The opening of the acrosomal vesicle exposes a still unidentified ligand (purple) that is bound by a complex of tetraspan protein complex in the egg cell membrane. (4) The mutual binding triggers the vesiculation of the membranes of the spermatozoon and the inner components of the sperm are released. The egg cell engulfs the nucleus, mitochondrion and centrioles of the sperm by endocytosis. (5) Mitochondria of the sperm are destroyed and degraded in the egg cell while the centrioles remain. (6) By means of exocytosis the cortical granules release enzymes (*blue*) that degrade the sperm-binding ZP-complex. A cortical reaction similar to that seen in the sea urchin egg and enzymatic linking of zona pellucida proteins provide a hardened fertilization envelope that hinders further sperm from entering the egg cell.