1. FERTILIZATION

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RECOMMENDED READING: Larsen's Human Embryology, 3rd Edition, pp.18-19.

SUMMARY: Fertilization is a cell-cell recognition process that occurs between two distinct cells: a small asymmetric and motile sperm cell and a large and nonmotile egg. The stages of fertilization can be divided into four processes: 1) sperm preparation, 2) sperm-egg recognition and binding, 3) sperm-egg fusion and 4) fusion of sperm and egg pronuclei and activation of the zygote. The specific structures of the sperm and egg that are important for fertilization will be discussed and experiments that led to the identification of the egg receptor for the sperm and the sperm receptor for the egg will be described. Membrane fusion of sperm and eggs is an incompletely understood process, but the discovery of proteins known as ADAM proteins on the sperm surface has suggested new mechanisms to explain sperm-egg fusion. Finally, we will consider how fertilized eggs prevent additional sperm from fusing (a condition known as polyspermy) and how the fertilized egg is activated to begin development.

LEARNING OBJECTIVES:

At the conclusion of the lecture you should be able to:

1. Discuss the sequential nature of fertilization in which ordered changes in the gametes "drive" the process of fertilization toward completion.
2. Explain the role of specialized sperm and egg surface structures in fertilization.
3. Describe how egg and sperm receptors were identified.
4. Explain the current state of knowledge about sperm-egg membrane fusion and how sperm components are incorporated into the egg.
5. Describe how polyspermy is prevented and the fertilized egg is activated for development.

GLOSSARY:

Capacitation: The process by which the sperm becomes capable of fertilizing an egg.
Acrosome Reaction: A regulated exocytotic event in which an apical vesicle in the sperm head fuses with the sperm plasma membrane. The acrosome reaction is triggered in response to egg factors.
Acrosin: A serine protease released during the acrosome reaction.
Cortical Reaction: A regulated exocytosis in which apically localized vesicles (cortical granules) in the egg fuse with plasma membrane after fertilization.
Zona Pellucida: A coat surrounding the egg that contains three glycoproteins.
Galactosyl transferase: An oligosaccaride modifying enzyme that is usually found in the Golgi but in sperm is on the cell surface. Thought to be important as the sperm receptor for the egg.
Fertilin: An ADAM family protein on the sperm implicated in sperm-egg membrane fusion. Contains a fusion peptide resembling viral fusion peptides and a disintegrin domain involed in recognition.

ADAM proteins: A family of proteins that contain A Disintegrin And Metalloprotease domain(s).
Pronuclei: The transitional male and female nuclei formed in the egg after fertilization. They fuse to form the diploid zygote nucleus.
Polyspermy: The condition in which more than one sperm fertilizes an egg. Polyspermy leads to defective development.

TEXT:

There are four stages to fertilization:

1. **Preparation: Capacitation** and **acrosome reaction**. Acrosomal vesicle fusion is the membrane fusion event of this stage.
2. **Binding:** Species-specific interaction of gametes.
3. **Fusion:** Merging of sperm and egg plasma membranes is the membrane fusion event of this stage.
4. **Activation (of the zygote): Cortical reaction** (fusion of cortical vesicles with the egg plasma membrane) and pronuclear fusion.

The two gametes involved:

Eggs:

Eggs are large (~100 μm), symmetrical and nonmotile cells (Fig. 1-1). Human eggs are arrested in metaphase of the second meiotic division and complete meiosis only upon fertilization. Their surface is covered by microvilli (Fig.1-2). Eggs are surrounded by a **zona pellucida** (Fig. 1-3), which is a glycoprotein coat composed of three glycoproteins (ZPGP I-III). All three of the glycoproteins contain O- and N-linked oligosaccharides (Fig.1-4). The zona pellucida is not an osmotic barrier (in fact, even virus are capable of penetrating it), however it is a barrier to the sperm. The zona pellucida is the species specific barrier to fertilization as shown by the hamster experiment. Human sperm are incapable of fertilizing intact hamster eggs, but can fertilize hamster eggs stripped of their zona pellucida. This is used clinically to assess the fertilizing capacity of sperm.

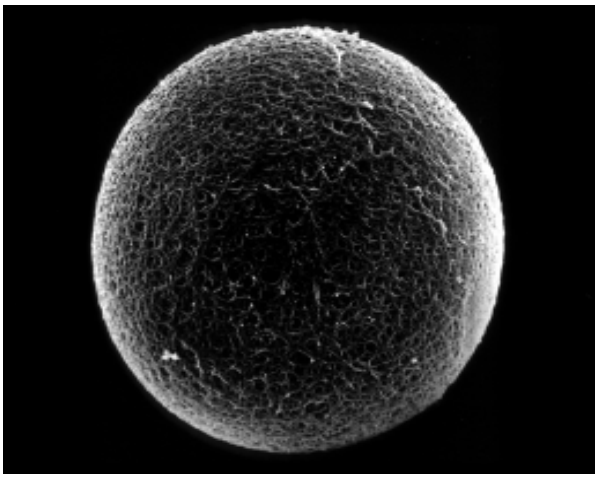


Fig. 1-1.

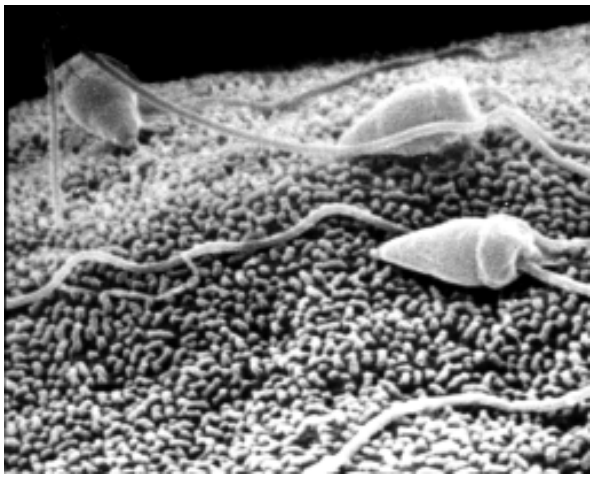


Fig. 1-2.

Sperm:

Sperm are small, asymmetrical and motile cells (Fig. 1-5). They have three components:
1. Tail: Also referred to as the principal piece. The tail contains the flagellar apparatus, which is composed of "9 + 2" microtubules and accessory structures (Fig. 1-6). The sliding of the microtubule is powered by the protein dynein. (Gibbons' movie of sliding microtubules)
2. Midpiece: at the proximal portion of the tail. Midpiece contains a sheath of mitochondria, which produce the ATP necessary for the beating of the tail.

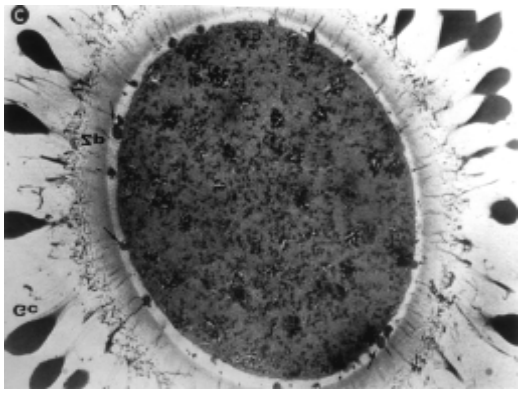
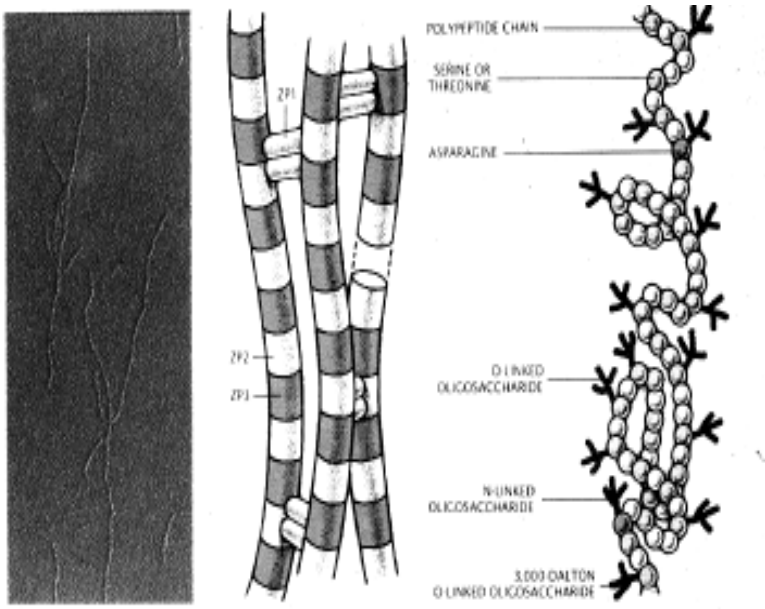


Fig. 1-3.



MOLECULE KNOWN AS ZP3 is a major component of the filaments (*left*) that associate to form the zona pellucida. ZP3 is a glycoprotein: a polypeptide to which sugar groups are attached. It combines with another glycoprotein, ZP2, to form the basic building block of the filaments, which are drawn highly schematically at the center; a third glycoprotein, ZP1, links the filaments. ZP3, shown in detail at the right, is the receptor molecule that binds sperm; it also induces the acrosome reaction. The actual binding elements are a subset of the sugar chains radiating from ZP3's polypeptide backbone; they are the O-linked oligosaccharides (those attached to the amino acids serine and threonine) with a molecular weight of about 3,900 daltons. The same sugar chains appear to collaborate with the polypeptide in ZP3 to induce the acrosome reaction.

Fig. 1-4.

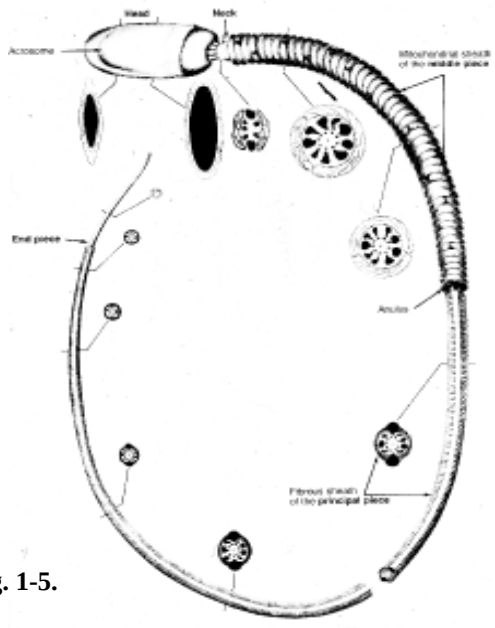


Fig. 1-5.

3. Head: contains the spermatic haploid nucleus. Overlaying the head is a membrane bound vesicle, the **acrosome**. Sperm do not possess any organelles associated with protein synthesis (Golgi, RER or lysosomes).

The sperm plasma membrane is also highly differentiated and contains proteins localized in distinct regions (Fig. 1-7). One of these, termed PH-30 or **fertilin**, is localized in the equatorial region of the sperm and is involved in sperm-egg plasma membrane fusion (see below).

Acrosome:

The acrosome is a lysosomal-like compartment derived from the Golgi. It has a low pH and contains soluble hydrolases (serine protease **acrosin**). In cross-section through the head of a sperm, one would cross four membranes in traversing from the plasma membranes to the nuclear membrane. During the **acrosome reaction**, fusion of the outer acrosomal membrane with the plasma membrane releases the contents of the acrosome and exposes the inner acrosomal membrane as the functional outer boundary of the sperm head.

The Four Steps of Fertilization:

Step I. Preparation of the Sperm.

Ejaculated sperm are not ready to fertilize an egg when they enter the vagina. In response to the dilution of semen in the vagina, they undergo several changes, which are collectively known as **capacitation**.

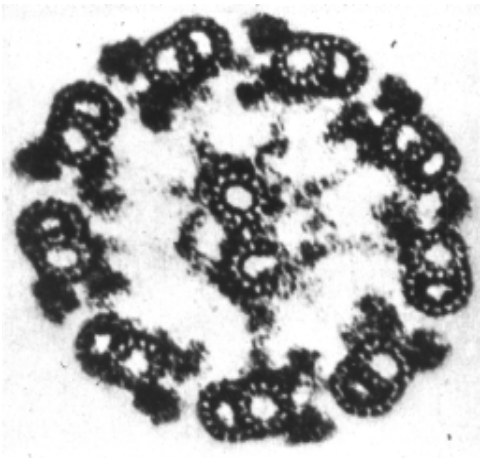


Fig. 1-6.

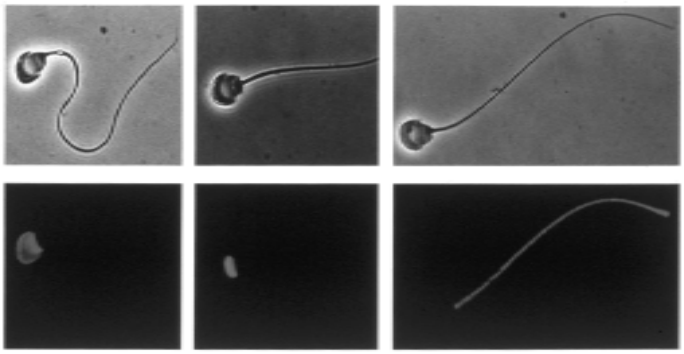


Fig. 1-7.

Fig. 1-8.

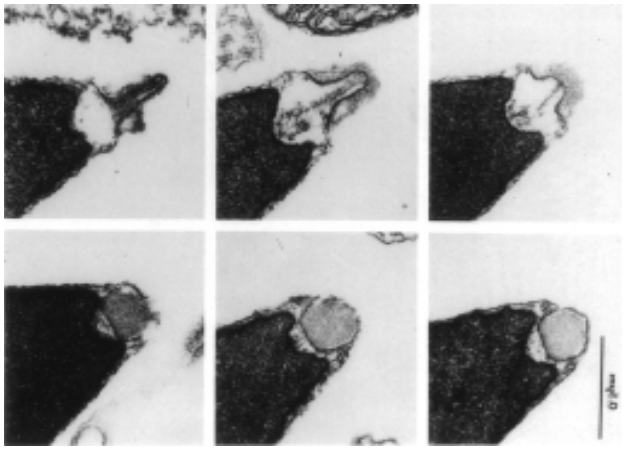


Fig. 1-9.

1. Intracellular Ca^{++} levels increase.
2. Spermatic motility is activated and tails change beat frequency.
3. Sperm cell surface antigens are lost. The loss of these proteins renders the sperm more receptive to binding to the egg.

Step II. Sperm-Egg Binding

Because of the availability of gametes, the process of sperm-egg binding was first studied and understood in invertebrates (Fig. 1-8). In sea urchins, the sperm head binds directly to the egg outer surface and this triggers the acrosome reaction.

(Figs.1-9 and 1-10). The acrosomal contents are released and there is a balanced Na^+ influx and H^+ efflux, causing an increase in pH. The increased pH triggers the dissociation of the profilactin complex (actin and profilin) and the released actin monomers polymerize to form a filament called the acrosomal process. This acrosomal process penetrates the egg coatings to allow fusion of the sperm and egg plasma membranes. In sea urchins then, the sperm literally skewers the egg.

In humans the process of sperm-egg binding is not so simple. The complicating factor is the thick zona pellucida, which keeps sperm from binding close to the egg plasma membrane.

Sperm receptor on egg.

Dr. Paul Wassarman used a **competition assay** to isolate and identify the factor in the zona pellucida that was involved in sperm egg binding (Fig. 1-11). Dr. Wassarman incubated sperm with zona pellucida glycoproteins (ZPGPs) he had isolated from unfertilized and fertilized eggs. He found that sperm preincubated with ZPGPs from unfertilized eggs were not able to fertilize eggs. Yet, when he

preincubated sperm with ZPGPs isolated from fertilized eggs, which are known not to bind sperm, the sperm could still fertilize eggs (Fig. 1-12 and 1-13). This showed that the isolated ZPGPs from unfertilized eggs contain a receptor for the sperm and that this receptor is modified after fertilization.

In follow up experiments, Dr. Wassarman purified ZPGP I, ZPGP II and ZPGP III and showed that only

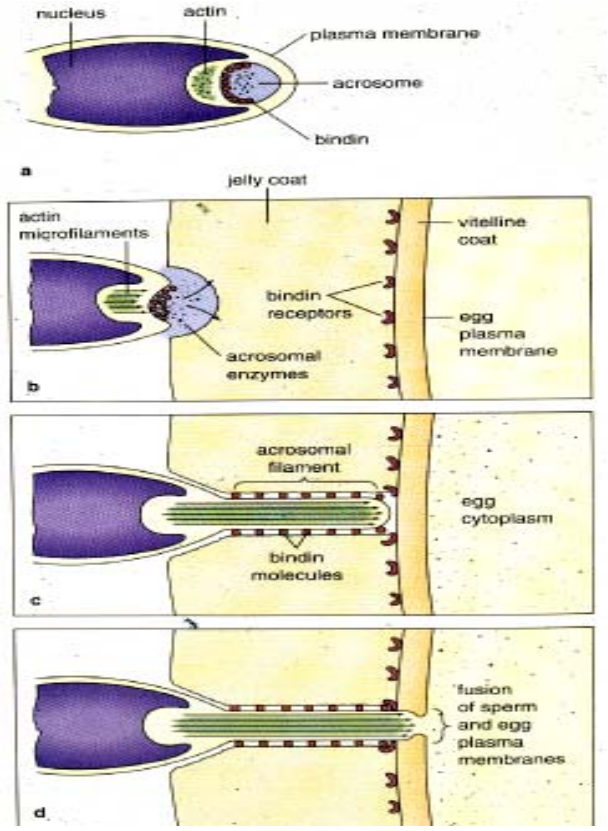


Fig. 1-10.

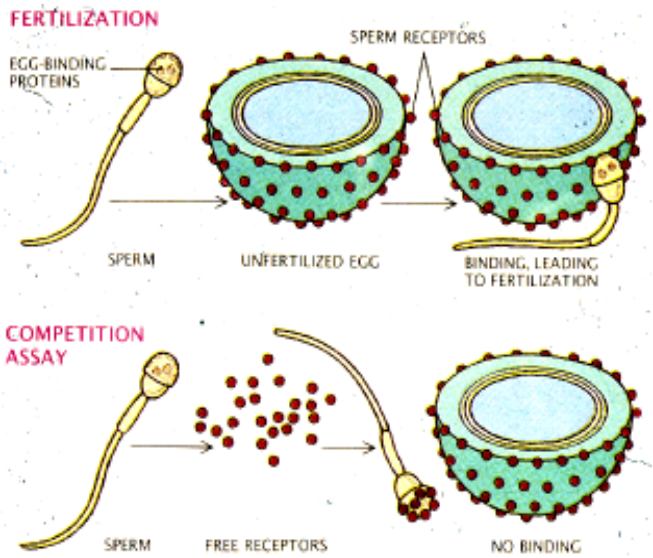


Fig. 1-11.

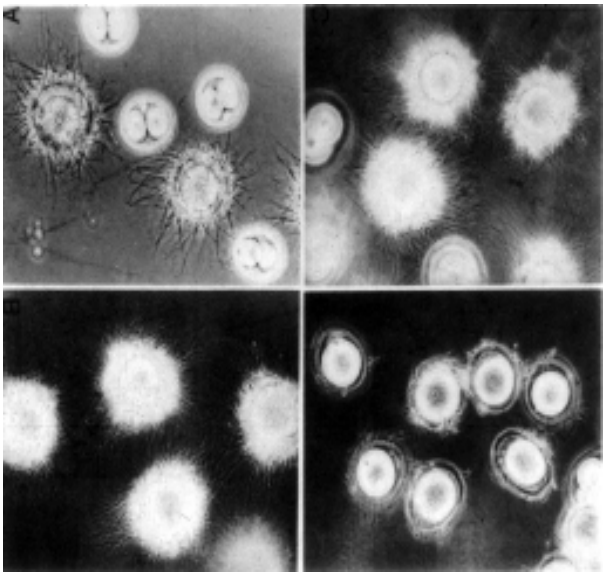
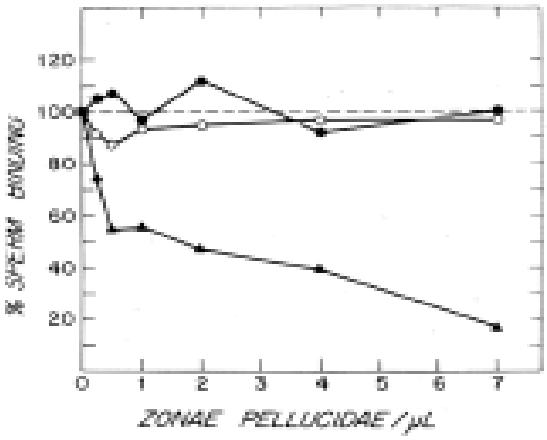


Fig.1-12.



Quantitation of Binding of Sperm to Eggs in the Presence of Various Concentrations of Zona Pellucida Proteins Purified from Zonae Pellucidae Isolated from Unfertilized Eggs

These experiments were carried out as described in the legends to Figures 1 and 2 using purified ZP1 (●), ZP2 (○) and ZP3 (▲) from zonae pellucidae isolated from unfertilized eggs. There were an average of 50 sperm bound per egg at the 100% binding level (■) and, in each case, a minimum of eight eggs were examined and included in the calculations.

Fig. 1-13

ZPGP III could prevent sperm binding to eggs showing that ZPGP III is the sperm receptor. By treating ZPGP III with agents that selectively hydrolyzed protein (trypsin), N-linked glycoproteins (specific glycohydrolase) and O-linked glycoproteins (weak base), Dr. Wassarman showed that the part of ZPGP III that was responsible for sperm binding was the O-linked oligosaccharide.

Egg receptor on sperm.

What sperm component is binding to the ZPGP III? Dr. Barry Shur was studying a Golgi enzyme known as **galactosyl transferase.** This enzyme catalyzes the addition of galactosyl residues from a donor nucleotide sugar, UDP-galactose, to the terminal end of O-linked oligosaccharides. As in all enzymatic reactions, there are two stages in catalysis:

1.The enzyme binds the substrates (in this case UDP-gal and O-linked oligosaccharide).
2.The enzyme catalyzes the reaction and releases the products (in this case, UDP and the modified O-linked oligosaccharide with galacosyl residues on its ends).

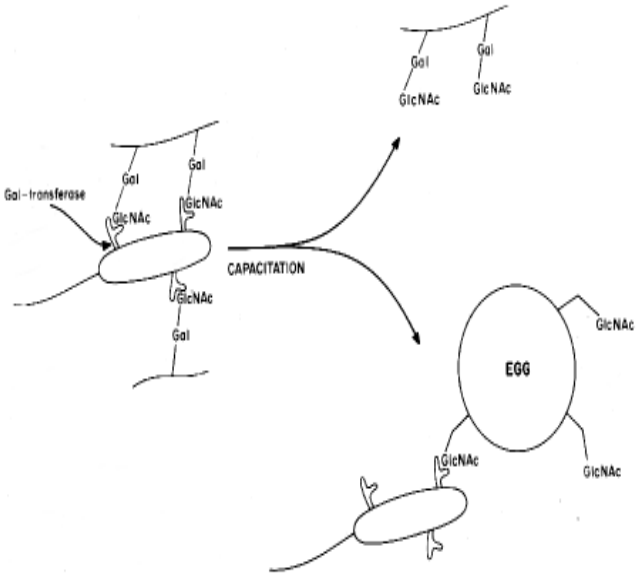


Fig.1-14

It is important to understand that if one of the substrates is not present, the enzyme may be able to bind the available substrate, but will not be able to catalyze the reaction. This is important in sperm binding.

Dr. Shur found that sperm, which have no Golgi apparatus, have galactosyl transferase on the surface of their plasma membrane. When sperm are ejaculated, they have oligosaccharides bound to the galactosyl transferase. During capacitation, these coating glycoproteins are removed, allowing the galactosyl transferase, to bind to other carbohydrates it may encounter, such as those attached to ZPGP III. The sperm that do encounter the egg and its zona pellucida, bind ZPGP III through their galactosyl transferases (Fig. 1-14). At this point, UDP-gal would normally bind to its site on galactosyl transferase, galactose residue would be transferred to the oligosaccharide and the modified oligosaccharide would be released. However, there is no high energy UDP-galactose in the extracellular fluid surrounding the egg so catalysis does not occur and the sperm remains tightly bound to the egg zona pellucida.

Many studies support a role for galactosyl transferase as the sperm protein involved in sperm-egg binding, however, other proteins may be involved. A recent genetic knockout of galactosyl transferase in mice yielded mice that were completely fertile and showed normal sperm-egg binding.

Acrosome reaction.

As a result of irreversible binding of the sperm to the egg, the zona pellucida triggers the acrosome reaction (Fig. 1-15). The outer plasma membrane of the acrosome fuses at multiple sites with the plasma membrane and the contents of the acrosome are released (Fig. 1-16). Two of the important components are **acrosin**, a serine protease, and **N-acetylglucoaminidase.** Acrosin bores a hole in the zona pellucida so that the sperm can reach the egg itself. N-acetylglucoaminidase hydrolyzes the O-linked oligosaccharides in ZPGP III to

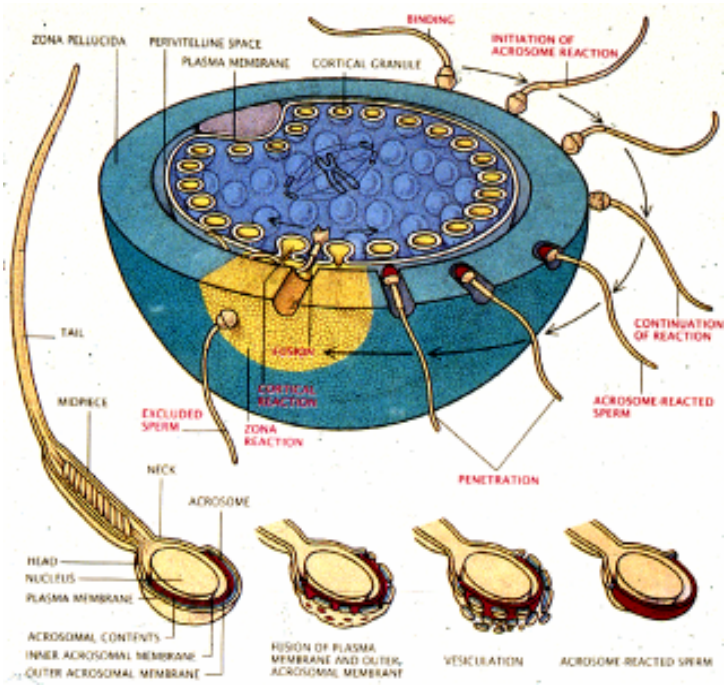


Fig. 1-15.

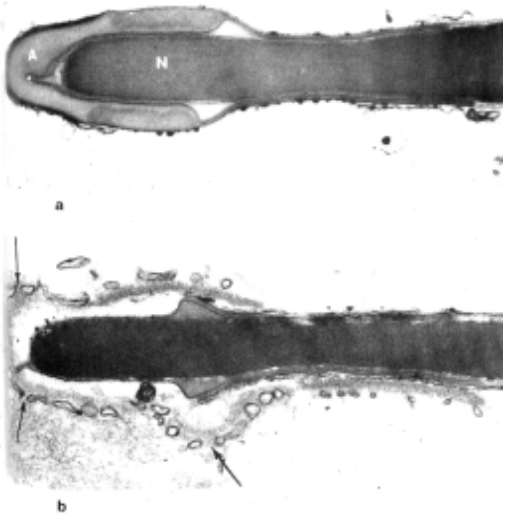


Fig. 1-16.

allow the sperm to detach. As a result of the membrane fusion, a new surface is exposed on the sperm (the inner acrosomal membrane) and this is thought to contain new binding sites for ZPGP II.

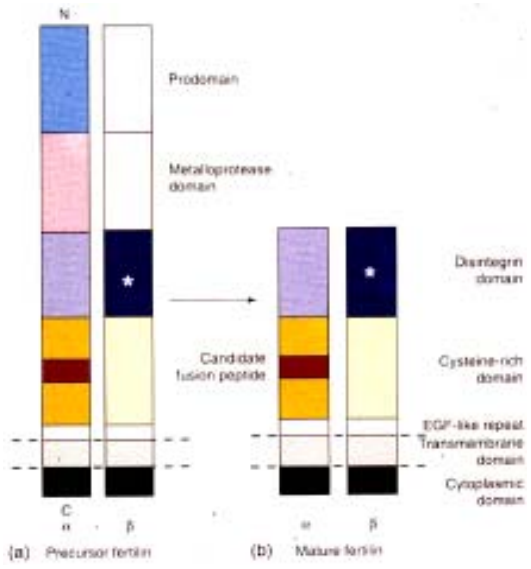


Fig. 1-17.

STEP III. Sperm-Egg Fusion.

For many years the process by which the plasma membrane of the sperm and egg fused was a black box. Recent studies by Drs. Judith White, Diana Miles, and Paul Primakoff and their colleagues, have now shed light on this process. Miles and Primakoff made an antibody to PH-30, a heterodimeric sperm membrane protein comprised of α and β subunits, and showed that this antibody blocked fertilization but did not block binding of sperm to eggs stripped of their zona pellucida. This suggested that PH-30 was involved in sperm and egg fusion and it was given the name **fertilin**.

Cloning and sequencing of fertilin revealed that the α-subunit had a hydrophobic domain that resembled those on viral proteins that are known to be involved in membrane fusion (Fig. 1-17 and 1-18). The β-subunit had a **disintegrin** domain. Disintegrins were first discovered in snake venom and act as competing ligands for integrins (for example, snake venom disintegrins will block platelet aggregation mediated by integrins). Both subunits had metalloprotease domains. Fertilin was one of the first proteins of a family of proteins known as ADAMs proteins (for A Disintegrin And Metalloprotease containing protein) that are involved in cell-cell recognition and cell fusion events. Although the mechanism for how fertilin causes sperm-egg membrane fusion is not known, studies have supported its role in membrane fusion (Fig. 1-19). For example, a peptide corresponding to the viral fusion peptide of

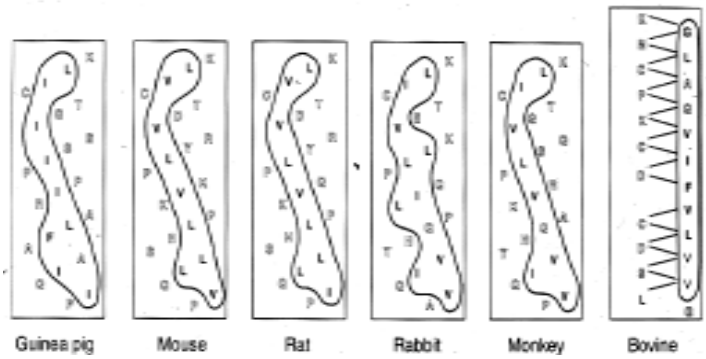


Fig. 1-18.

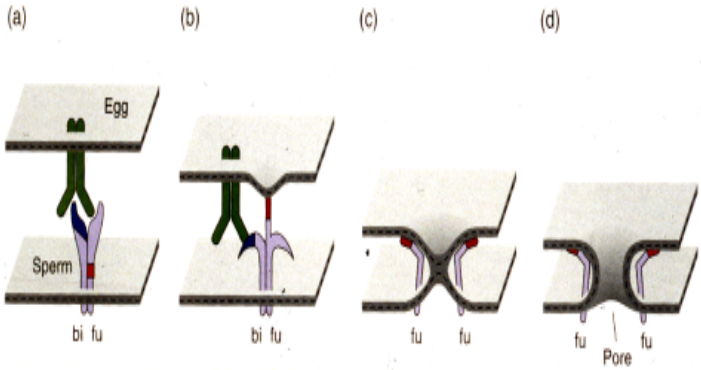


Fig. 1-19.

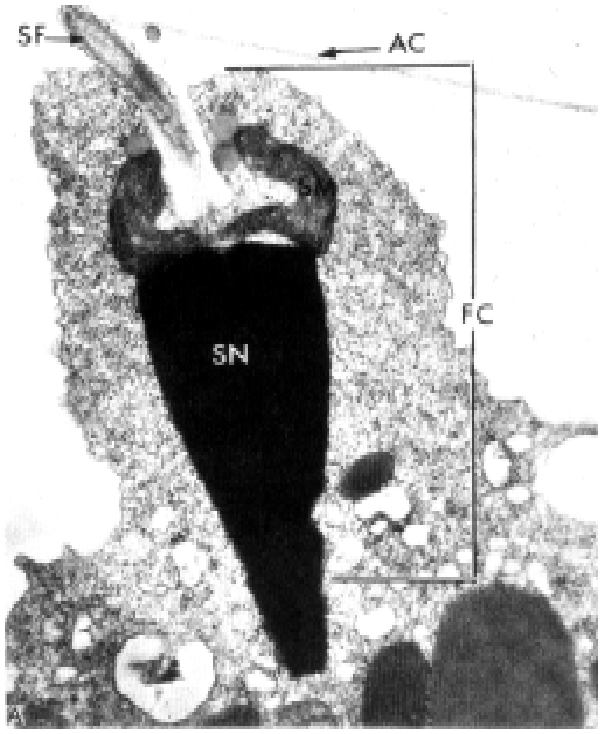


Fig. 1-21.

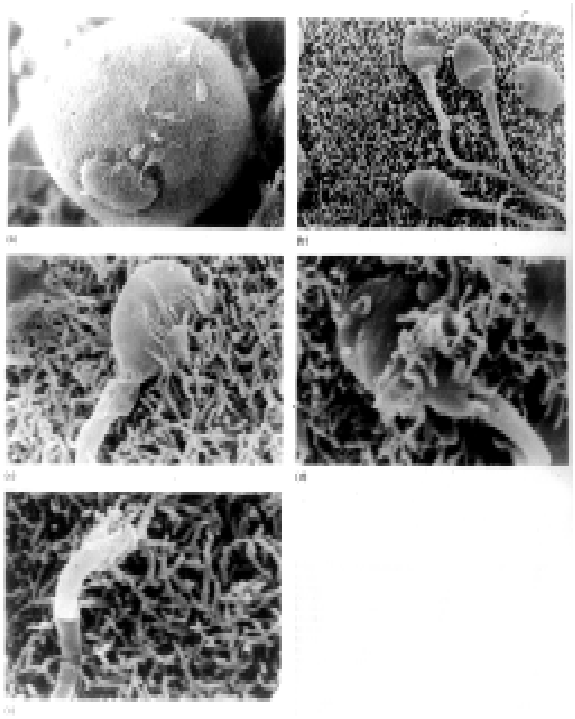


Fig. 1-20.

α-fertilin is capable of fusing model membrane vesicles and the disintegrin domain of β-fertilin will block sperm-egg fusion. The egg integrin involved in sperm-egg fusion (the receptor for the β-subunit disintegrin) is known to be α6β1.

Once the sperm fuses with the egg**,** the beating of the tail stops immediately. The sperm instead is drawn into the egg by elongation and fusion of the egg's microvilli (Fig. 1-20). As a result, the sperm nucleus and other organelles are incorporated into the egg cytoplasm (Fig. 1-21). The sperm nucleus undergoes a series of changes, including chromatin decondensation and formation of a new nuclear envelope, to form a male **pronucleus** (Fig. 1-22). The male pronucleus uses microtubules to migrate to the center of the cell, where it fuses with the female pronucleus to reconstitute a diploid nucleus. Other sperm organelles (e.g., mitochondria) persist during early cleavage stages of the embryo and it is conjectured that they may play a role in development (Figs. 1-23 and 1-24).

STEP IV. Activation - The Egg's Response.

The immediate events after fertilization include the egg's effort to prevent **polyspermy**. Polyspermy refers to the fertilization of the egg by more than one sperm, resulting in zygotes with greater than a diploid amount of DNA. This causes early embryonic defects and arrest of development.

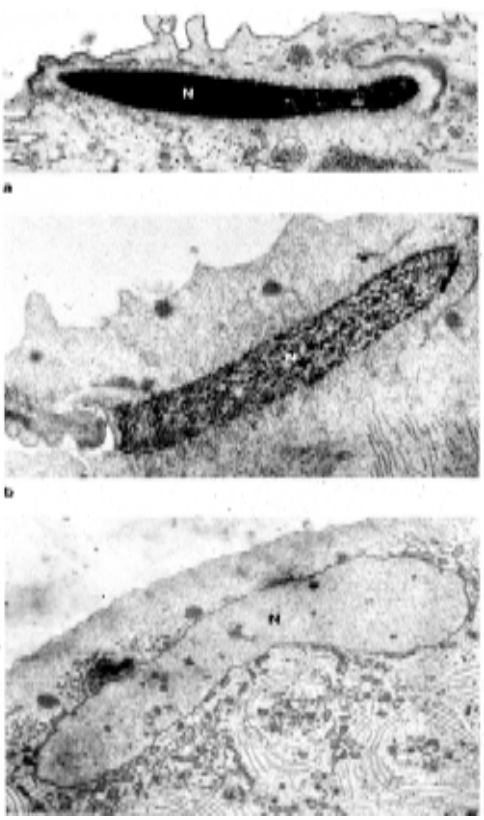


Fig. 1-22.

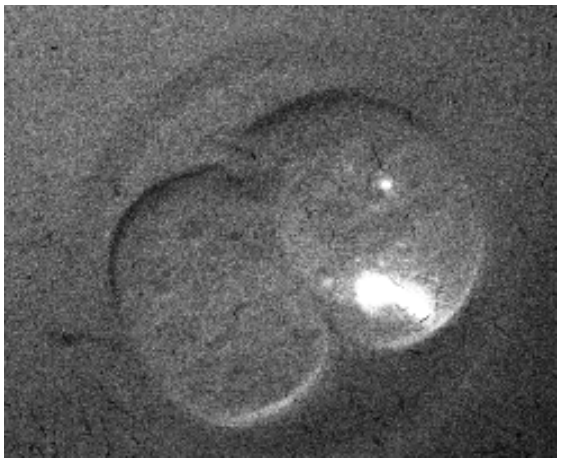


Fig. 1-23.

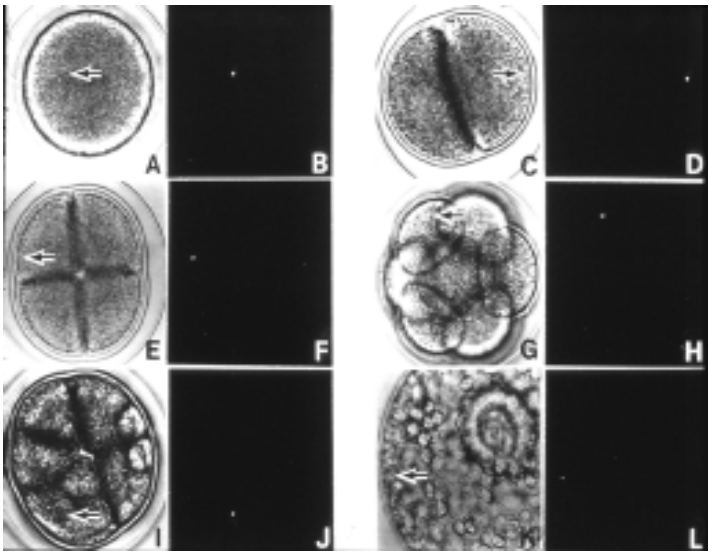


Fig. 1-24.

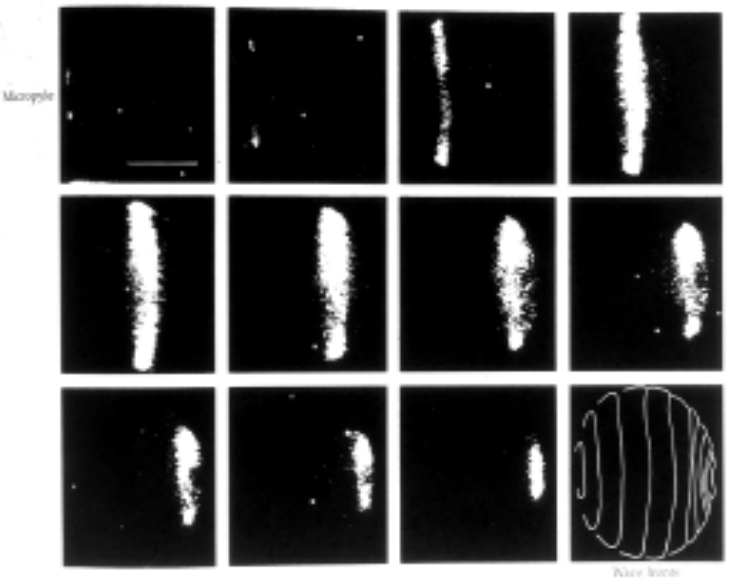


Fig. 1-25.

After sperm-egg fusion, the egg mounts the **cortical reaction** to prevent polyspermy. In all eggs, residing just under the plasma membrane there are membrane bound vesicles known as **cortical granules**. When a single sperm penetrates the egg, the cortical granules adjacent to the site are triggered to fuse with the plasma membrane, exocytosing their contents into the perivitelline space (the space between the plasma membrane and the zona pellucida). The cortical reaction is propagated over the surface of the egg by a wave of Ca^{++}. This was shown by the aequorin experiment in which the photoprotein aequorin phosphoresced in a wave from the site of sperm penetration of the egg (Fig. 1-25 and movie).

As a result of the cortical reaction, two important enzymes are released into the perivitelline space:

1. **Ovoperoxidase**: In sea urchins, ovoperoxidase catalyzes the crosslinking of tyrosine residues in the extracellular matrix. This makes the extracellular matrix tough and insoluble (analogous to the tanning of leather) and a physical barrier is formed which prevents other sperm from fertilizing the egg. In mammals, ovoperoxidase does not catalyze tyrosine cross-linking to the point of insolubility. In mammals, its major effect is thought to be as a spermicial agent.

2. **Hydrolase**. Remember Wassarman's result showing that zona pellucida from fertilized eggs was incapable of blocking fertilization? Another cortical granule that is released is a specific hydrolase, which degrades O-linked oligosaccharides on ZPGP III. This renders the zona pellucida incapable of binding additional sperm, thus preventing polyspermy.

Activation of the egg also includes the initiation of development of the new zygote. Protein synthesis and other metabolic processes are upregulated to provide for the developing embryo.