

Fertilization

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Fertilization is a complex process which involves the fusion of male and female gametes followed by the fusion of their cytoplasm. Fertilization begins with the sperm's approach to the egg and ends with the fusion of egg and sperm pronuclei. It involves a series of events. The process of fertilization has dual independent functions (i) to cause the egg to start developing i.e. **Activation**, and (ii) to inject a male haploid nucleus into the egg cytoplasm, i.e. **Amphimixis**.

Activation involves (i) the completion of second maturation division, if not occurred earlier; (ii) the egg cortex to form fertilization membrane outside the plasma membrane; (iii) the egg cytoplasm to start various metabolic reactions; and (iv) stimulation of mitosis for cleavage by contribution of sperm's centriole to the egg. **Amphimixis** is the most important event occurring in the life of all animals and plants and involves (i) the intermingling of paternal and maternal hereditary characters in the offspring; (ii) restores the diploid state of animal cell; and (iii) includes genetic variations in the species. The genetic variations provide the raw materials to the natural selection for speciation (species formation).

The process of fertilization has been studied mostly in marine invertebrates, such as, sea urchins, polychaetes, echinoids etc. whose eggs are typically translucent and without much yolk. Among vertebrates most fertilization studies have been done in amphibians and mammals. Attention has been focussed on amphibians because they are available and suitable for analytical and experimental procedures, and on mammals because man himself is a mammal and the need to know what happens is great.

Basic Requirements of Fertilization

In most animals the act of fertilization requires the following fundamental conditions for its fulfilment :

- (1) In most animals fertilization requires a fluid medium which may be sea water in marine forms, fresh water in fresh water forms or some body fluid in viviparous animals.
- (2) The life span of gametes is limited. Mature eggs quickly "over ripe", they must be activated promptly if they are to be activated at all. Eggs that are shed into the water like those of most invertebrates, fish and amphibians have shorter life span while those fertilized within the body of the female generally have a longer life span; for example, the human egg can be fertilized for atleast 24 hours after ovulation. Sperms are also short lived, but there are many exceptions to this rule in nature. For example, in bat viable sperms may persist in the female reproductive tract for many months in the winter, during hibernation. Further, life span of human sperm in female genital tract is 24 hours.
- (3) To increase the probability of fertilization, the number of sperms must exceed the number of eggs. In mammals a single ejaculation may range from 0.05 ml in bat, 2 ml in rat, 3 ml in man to 500 ml in boar containing 6 million, 2 to 5 million, 1 million cells per microliter respectively.

Mechanism of Fertilization

The process of fertilization completes in the following five stages :

- (1) Encounter of spermatozoa and ova.
- (2) Capacitation and contact.
- (3) Acrosome reaction and penetration.
- (4) Activation of ovum.
- (5) Migration of pronuclei and amphimixis.

[I] Encounter of spermatozoa and ova

A major problem in sexual reproduction is how to bring together the spermatozoa and ova in the same locality in a fluid medium, so that individual sperms may reach the surface of ova at the right time. Previously it was thought that sperms are attracted towards the ripe eggs by chemotaxis. A chemical substance is found in the cortex of the eggs of sea urchin *complanularia*, fishes etc. held responsible for the attraction of sperms to ripe eggs.

In general, most invertebrates, and vertebrates accomplish close approximation of spermatozoa and eggs through special devices or particular forms of behaviour. The primary needs for the encounter of spermatozoa and ova are fluid medium for the act of fertilization and delivery of large quantities of spermatozoa close to the numbers of ripe

eggs at the right time. According to the place and nature of fluid media following two kinds of fertilization have been reported :

- (1) External fertilization
- (2) Internal fertilization

1. External fertilization. This type of fertilization occurs in liquid medium outside the bodies of parent animals. Among fresh water animals (fishes, amphibians and fresh water invertebrates), the timing of spawning of egg by the female and shedding of sperms by the male parent are very specific. As their spermatozoa remain active usually for a few minutes, the sperms are delivered directly to the eggs of an individual female immediately after egg laying. But marine forms shed eggs and sperms freely into the surrounding water. The time interval between the laying of eggs and the shedding of sperms may even be weeks or months, because the saltish sea water serves as an important physiological medium for gametes.

During external fertilization, the movement of spermatozoa in a liquid medium is entirely at random and the spermatozoa collide with the eggs as a matter of chance which occurs regularly in nature, partly due to enormous number of spermatozoa produced by the male and partly because the eggs being relatively larger targets, they can be hit by sperms fairly well.

2. Internal fertilization. In oviparous forms such as reptiles and birds, the eggs are completely enclosed in impermeable egg membranes or they are retained within the maternal body throughout development in ovoviviparous and viviparous animals. In all such cases the spermatozoa are delivered internally in the body of the female by some type of copulatory mechanism or by **intromittant organ** of the male. In such forms the fertilization may occur in the lower part of the oviduct (eg. *Urodela*), in the upper portion of oviduct (eg. salamanders, reptiles, birds and mammals) or in the ovarian follicles in viviparous fishes (e.g. *Gambusia affinis*) and eutherian mammals (e.g. *Ericulus*).

In terrestrial animals, there is no problem of timing of the spawning of eggs and shedding of sperms because the mature sperms are commonly stored in a physiological medium capable of maintaining their life and potential activity for days or even for months, either in moisture conserving capsules or in compartments of male and female body to be picked up, transferred or utilized in one way or another. The movement of the spermatozoa from the site of deposition to the site of fertilization usually depends on the active swimming of the spermatozoa themselves or transported passively by muscular contractions of the female tract and also

by the counter currents in the cilia which propel backward flowing liquid content of the tract.

[III] Capacitation and contract

In some animal species, the spermatozoa undergo a process called **capacitation**, before they are fully capable of fertilizing an egg of the same species or the change in spermatozoa which makes it capable of fertilizing the egg has been called **capacitation**. The phenomenon of capacitation relates to the specificity of fertilization, namely, the capacity of spermatozoa to fertilize eggs of the same species but not of other species.

Frank Lillie was the first to show that a chemical substance is discharged by the newly laid sea urchin egg. This substance diffuses into the sea water and causes sperm cells in the neighbourhood to become more active and attracted to the cell. This condition is called as **chaemotaxis**, implying that the sperms were responding to this chemical substance. This substance has been found in the jelly which surrounds the eggs.

1. Agglutination. In most animals it has been observed that in the presence of ripe eggs or even the water in which ripe eggs of the same species have been lying for sometime, the spermatozoa adhere to the surface of the egg by its lateral side and even to each other. This reaction is usually visible within a few seconds and the spermatozoa are seen to clump together head to head or less commonly tail to tail. This adhesion of spermatozoa results in their **clumping** or **agglutination**. It depends to a large extent on the environmental conditions.

2. Fertilizin-antifertilizin reaction. The cause of agglutination of spermatozoa was studied by Lillie (1919). He observed that **fertilizin** and **antifertilizin** occur in the eggs and sperms respectively. They are directly involved in the reaction between the egg and the spermatozoon. The main source of fertilizin is the egg itself and it is located in the plasma membrane. However, in the eggs of sea urchin and other echinoderms, it is produced by the layer of jelly surrounding the egg, and becomes accumulated in the external gelatinous coat.

The fertilizin is a gel formed of glycoprotein or mucopolysaccharide. As a protein, it contains a number of aminoacids and as a polysaccharide it includes molecules of one or more monosaccharides. The monosaccharides glucose, fructose, fucose or galactose are esterified by sulphuric acid as shown in the formula in fig. 4-1.

Both the aminoacids and polysaccharides vary from one species to another, that is why each species possesses its specific type of fertilizin. The

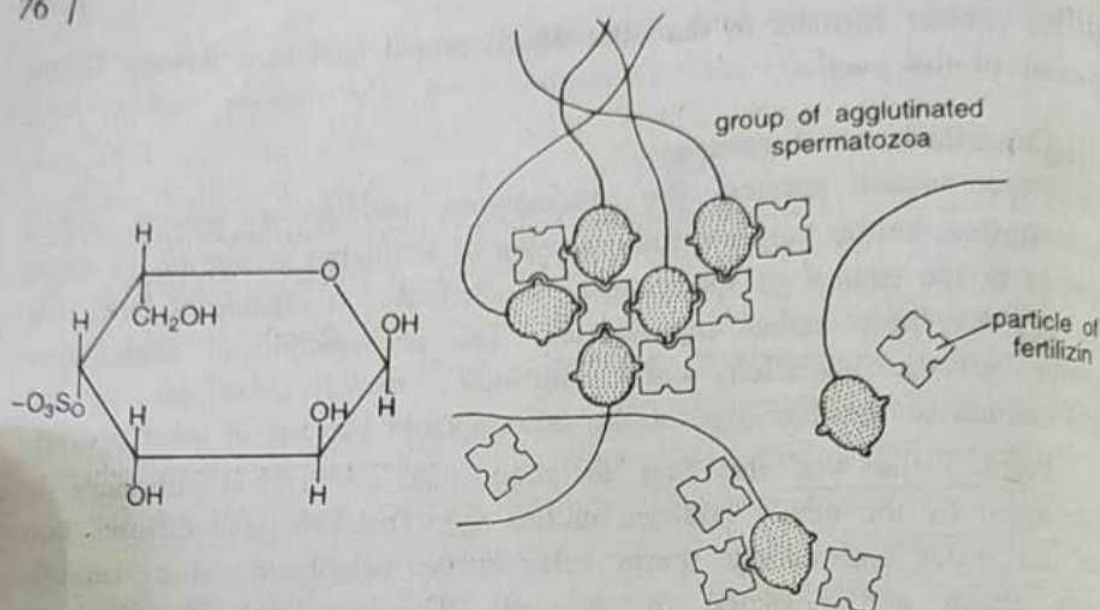


Fig. 4-1. Agglutination of sperms.

molecules of fertilizins are quite large—the molecular weight is about 300,000—and each molecule may have more than one “active group”, so that one fertilizin particle may become attached to two or more spermatozoa thus binding them together.

The surface layer of cytoplasm of spermatozoa (i.e. sperm plasma membrane) contains another species specific acid protein known as **antifertilizin** (or antifertilizins). The antifertilizins can be extracted from the spermatozoa by heating, freezing and thawing, or acidifying the water.

The remarkable peculiarity of the fertilizins and antifertilizins is that they combine in a specific manner; that is, the egg fertilizin of a particular species reacts best with the sperm antifertilizin of the same species. Reactions between the species which are nearly related to each other are very much weaker. The reaction between fertilizin and antifertilizin is very much similar to the reaction between antigen and antibody. In both cases, a “chemical lock” is formed between two complementary substances. Thus during capacitation and contact stage of fertilization when spermatozoa and eggs of same species come in physical contact of each other, a chemical lock is established between the antifertilizin molecules of spermatozoa and fertilizin molecules of unfertilized egg and due to the fact many spermatozoa adhere to the surface of an unfertilized egg.

It has been suggested that the main function of fertilizin-antifertilizin is to thin out the number of spermatozoa around the egg, so that the chances of two or more spermatozoa fusing with the egg at the same time are diminished.

[III] Acrosome reaction and penetration

As mentioned earlier, except porifers and coelenterates, where the surface of the ripe egg is seldom naked most animal eggs are enveloped by one or more egg membranes or gelatinous layers or follicle cells or both, outside the plasma membrane. These layers constitute barriers for the penetration by spermatozoa and serve in preventing fertilization by more than one spermatozoon or by sperm of other species.

When a spermatozoon is attached to surface of the egg, it becomes motionless. Its penetration through egg membranes and also through the plasma membrane of the egg is achieved by some physiochemical activity of the sperm acrosome. Certain enzymatic proteins called **sperm lysins** are produced presumably by the sperm acrosome.

The sperm lysins differ from one animal group to another. In some cases the dissolution of the egg envelopes may be brought about by simpler means. Thus, it is believed that the jelly coat of echinoderm eggs may be dissolved as a result of acidification of seawater by carbondioxide produced by the spermatozoa in the course of their respiration. In the case of eggs with very thick and resistant envelopes, such as the egg envelopes of fishes and insects, the sperm cannot reach the egg at all points but must penetrate through a special canal, the **micropyle**, left in the egg envelope, the chorion. In mammals, when the eggs are released from the ovary, they are commonly encased in a layer of follicular cells, called **corona radiata**. These cells are held together by an adhesive cementing substance called **hyaluronic acid**, a mucopolysaccharide. The corona radiata, thus, acts as a barrier through which the spermatozoon must first penetrate to reach the plasma membrane of the egg. For this purpose, the sperm's acrosome produces an enzyme, **hyaluronidase**, which serves to dissolve the adhesive substance and disperses the cells of corona radiata. The breaking of the membranous barriers is not only mediated by lytic agents provided by the acrosome of the spermatozoon, but the acrosome itself undergoes morphological changes and forms acrosomal filament which helps the sperm penetration into the egg interior. The entire process has been well illustrated in echinoderms, annelids and *Saccoglossus*.

Acrosome reaction and penetration in *Saccoglossus*. Penetration and acrosomal reaction of spermatozoa of *Saccoglossus* has been best described by Colwin and Colwin (1967). A spermatozoon of *Saccoglossus* has spherical nucleus, a flat tail and an acrosomal vesicle at the anterior end of the sperm head.

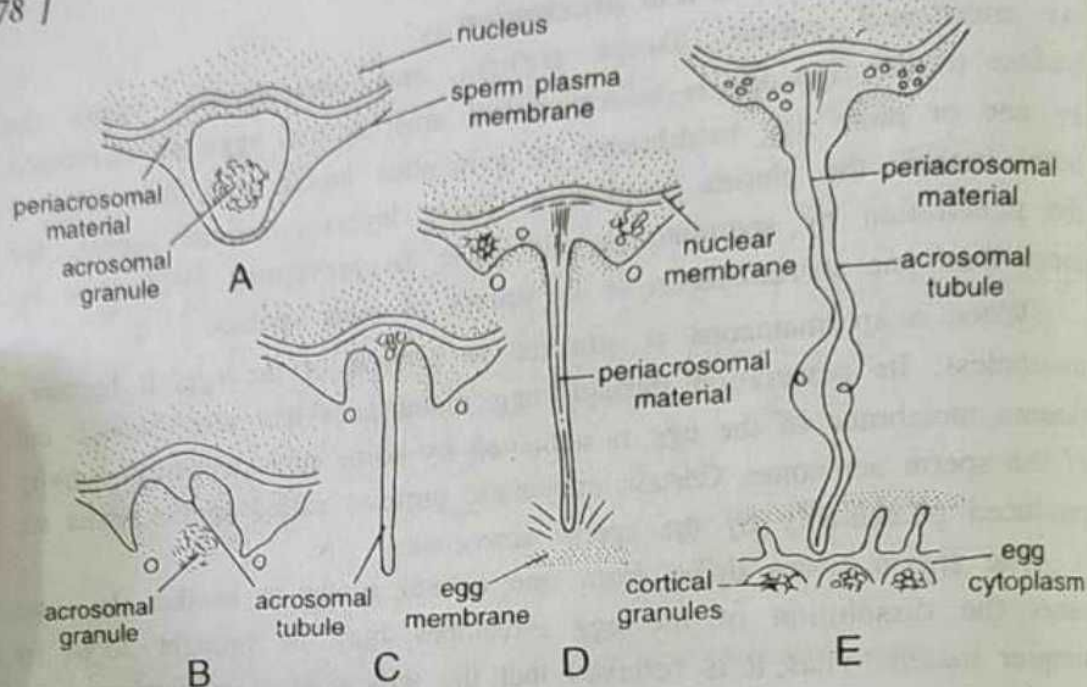


Fig. 4-2. Changes in the spermatozoon during fertilization in *Saccoglossus* A—Acrosome of inactivated spermatozoon; B—Extrusion of acrosomal granules; C—Formation of acrosomal tubule; D—Acrosomal tubule reaches egg membrane; E—Acrosomal filament reaches the surface of egg cytoplasm.

The acrosomal vesicle is bounded by an acrosomal membrane and contains a large, dense acrosomal granule. The granule is surrounded in large part by fine, grainy material except at the apex where an apical space lies between the granule and membrane. The space between the acrosomal membrane and sperm plasma membrane and also, the space between acrosomal membrane and nuclear membrane, are filled by some material called **periacrosomal material**.

As the spermatozoon of *Saccoglossus* makes its initial contact with the egg envelope, the following events occur.

(a) **Bursting of acrosome.** Apex of the acrosome bursts so that the membrane of sperm and acrosome open apically and consequently expose the interior of the acrosomal vesicle to the outside. The two membranes join around the margin of the opening.

(b) **Release of lytic enzymes.** Acrosomal granule is released and comes in contact with the egg envelope. It contains lytic enzymes which make passage through the egg envelopes. Therefore, shortly after its release, the acrosomal granule disintegrates and disappears.

(c) **Formation of acrosomal tubule.** Shallow depression of the acrosomal membrane close to the nucleus now deepens and soon lengthens into a

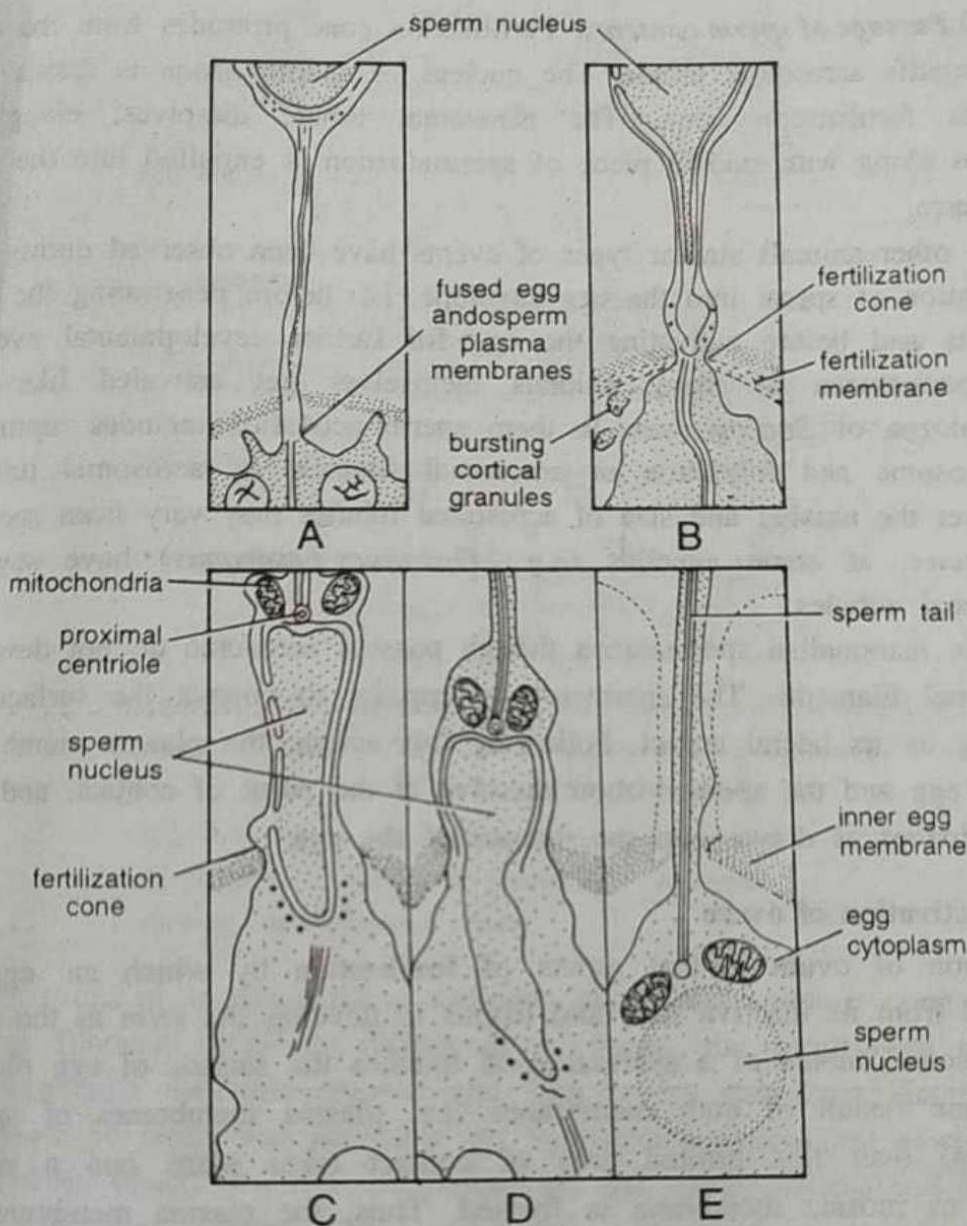


Fig. 4-3. Spermatozoon of *Saccoglossus* penetrating into the egg cytoplasm.

long slender **acrosomal tubule**. The tubule becomes twice as long as the sperm nucleus.

(d) **Eversion of the acrosomal membrane.** Rest of the acrosomal membrane everts and is added to the acrosomal tubule at its base. This is simply an unfolding of the already continuous membrane.

(e) **Fusion of acrosomal tubule with egg membrane.** Acrosomal tubule gradually enters through the passage of egg envelope, which has been made previously by acrosomal lytic enzymes, and ultimately touches and fuses with the egg plasma membrane.

(Z-22)

(f) *Passage of sperm contents.* Fertilization cone protrudes from the egg and engulfs acrosomal tubule. The nucleus of spermatozoon is drawn out towards fertilization cone. The acrosomal tubule dissolves; elongated nucleus along with middle piece of spermatozoon is engulfed into the egg cytoplasm.

In other animals similar types of events have been observed during the penetration of sperm into the egg envelope, i.e. before penetrating the egg contents and before activating the egg for further developmental events, the spermatozoa of many animals themselves get activated like the spermatozoa of *Saccoglossus*. In them sperm activation includes rupturing of acrosome and formation of acrosomal filament or acrosomal tubule. However the number and size of acrosomal tubules may vary from species to species, as some annelids (e.g., *Hydroides hexagonus*) have several acrosomal tubules.

The mammalian spermatozoa though possess acrosome do not develop acrosomal filaments. The spermatozoon appears to contact the surface of the egg by its lateral aspect. Following this action, the plasma membrane of the egg and the spermatozoon dissolve at the point of contact, and the spermatozoon is drawn into the interior of the egg.

[IV] Activation of ovum

Activation of ovum is that aspect of fertilization by which an egg is released from its inactive state and begins to develop. As soon as the apex of acrosomal tubule of a spermatozoon touches the surface of egg plasma membrane fusion of both membranes (i.e. plasma membranes of sperm and egg) over this limited area of contact takes place and a single continuous mosaic membrane is formed. Thus, the plasma membrane of both gametes (sperm and ovum) becomes continuous and forms a single cell, called **zygote**. At this very time, certain very important changes occur in the cytoplasm of egg :

- (1) Fertilization cone formation.
- (2) Cortical reactions and formation of fertilization membrane.
- (3) Metabolic activation.

1. **Fertilization cone formation.** Immediately after the acrosomal filament of spermatozoon touches the surface of the egg, the cytoplasm of the egg bulges forward at the point of contact, producing a process of hyaline cytoplasm called the **fertilization cone**.

Fertilization cone develops or appears in many forms. It may be in the form of a more or less simple conical protrusion or it may consist of several irregular pseudopodium-like processes, or in some cases it may

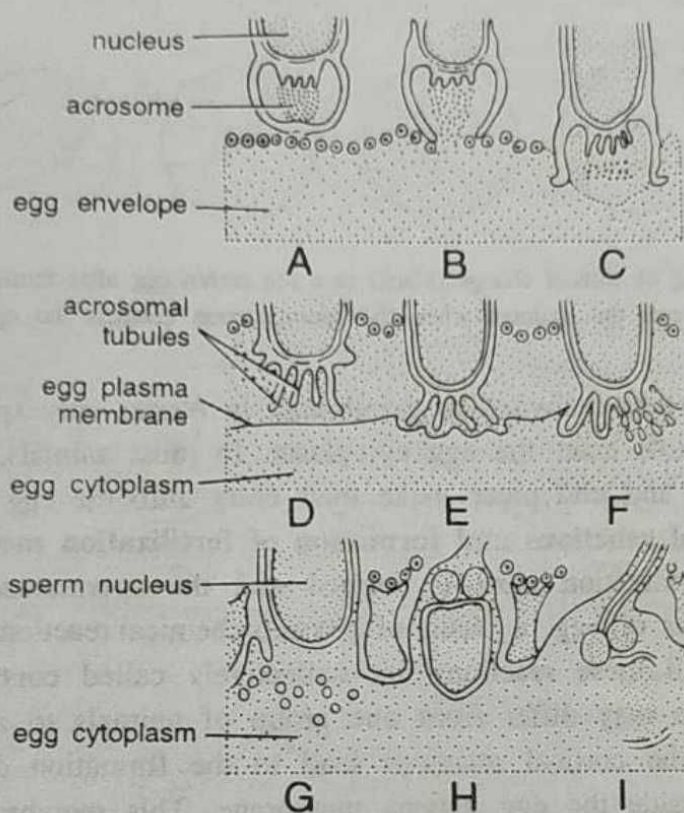


Fig. 4-4. Stages of sperm-egg association during fertilization of polychaete (Annelida), *Hydroides*, showing many acrosomal tubules.

take the form of a cytoplasmic cylinder stretching forward along the acrosomal filament or tubule. Whatever its shape, the fertilization cone gradually engulfs the spermatozoon and then begins to retract. Here one point should be clear, that normally the spermatozoon does not enter the egg cytoplasm intact, nor, it is swallowed but the sperm nucleus and other sperm structures (periacrosomal material, proximal centriole and mitochondria of mid-piece of spermatozoon) pass to the fertilization cone of the egg. The plasma membrane of sperm becomes one entity of plasma membrane of the egg.

Further during the whole penetration process of sperms into the egg, acrosomal granule never makes its entry into the egg, but only the periacrosomal material is injected into the egg cytoplasm along with other contents of the sperm. Some workers suggest that this periacrosomal material is responsible for the activation of egg.

There exists some variation in different animals, as to how much of the spermatozoon is taken into the interior of egg during fertilization. In mammals, complete structures of spermatozoon (viz., nucleus, mid piece, tail etc.) penetrate into the egg cytoplasm. In echinoderms, sperm tail

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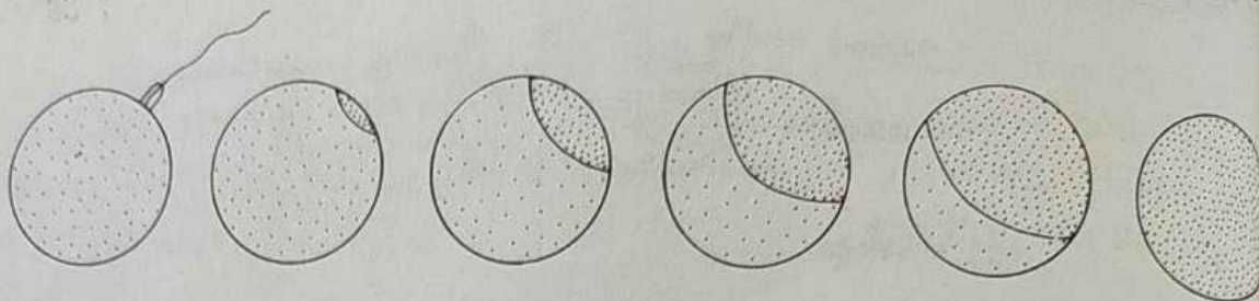


Fig. 4-5. Spreading of cortical change (black) in a sea urchin egg after fertilization. The figure at the left represents the moment when the spermatozoon contacts the egg surface.

remains exterior to the vitelline membrane; in *Neries* only sperm head and proximal centriole enter the egg cytoplasm. In most animals, however, the sperm nucleus and mid piece make their entry into the egg as a rule.

2. Cortical reactions and formation of fertilization membrane. Even before the fertilization cone is formed and the spermatozoon penetrates into the interior of egg, a chain of physico-chemical reactions is set in the egg cortex. All these reactions are collectively called **cortical reactions**. These reactions may differ from one group of animals to another, but in most groups, the cortical reactions lead to the formation of **fertilization membrane** outside the egg plasma membrane. This membrane blocks the entry of the late arriving spermatozoa in the egg interior, and thus avoids polyspermy. The process of cortical reactions and fertilization membrane formation in different groups of animals is as under :

(a) *Sea urchins*. In sea urchins, as soon as the apical end of acrosomal tubule touches the surface of egg, from the site of contact, a wavelike color change from yellow to white (under dark field microscopy) travels rapidly around the egg cortex and is shortly followed by the elevation of fertilization cone from the egg surface and the formation of fertilization membrane around the egg plasma membrane.

Electron micrographs of sea urchin's unfertilized eggs show that the egg cortex is bounded by two membranes (i) an outer 30 \AA° thick, vitelline membrane, and (ii) an inner, 60 \AA° thick plasma membrane.

Beneath the plasma membrane occurs a layer or cortical granules. A fertilization membrane is formed in the following stages :

The outer vitelline membrane separated from the plasma membrane undergoes expansion and becomes the outer layer of the fertilization membrane. The cortical granules explode and release the following three components :

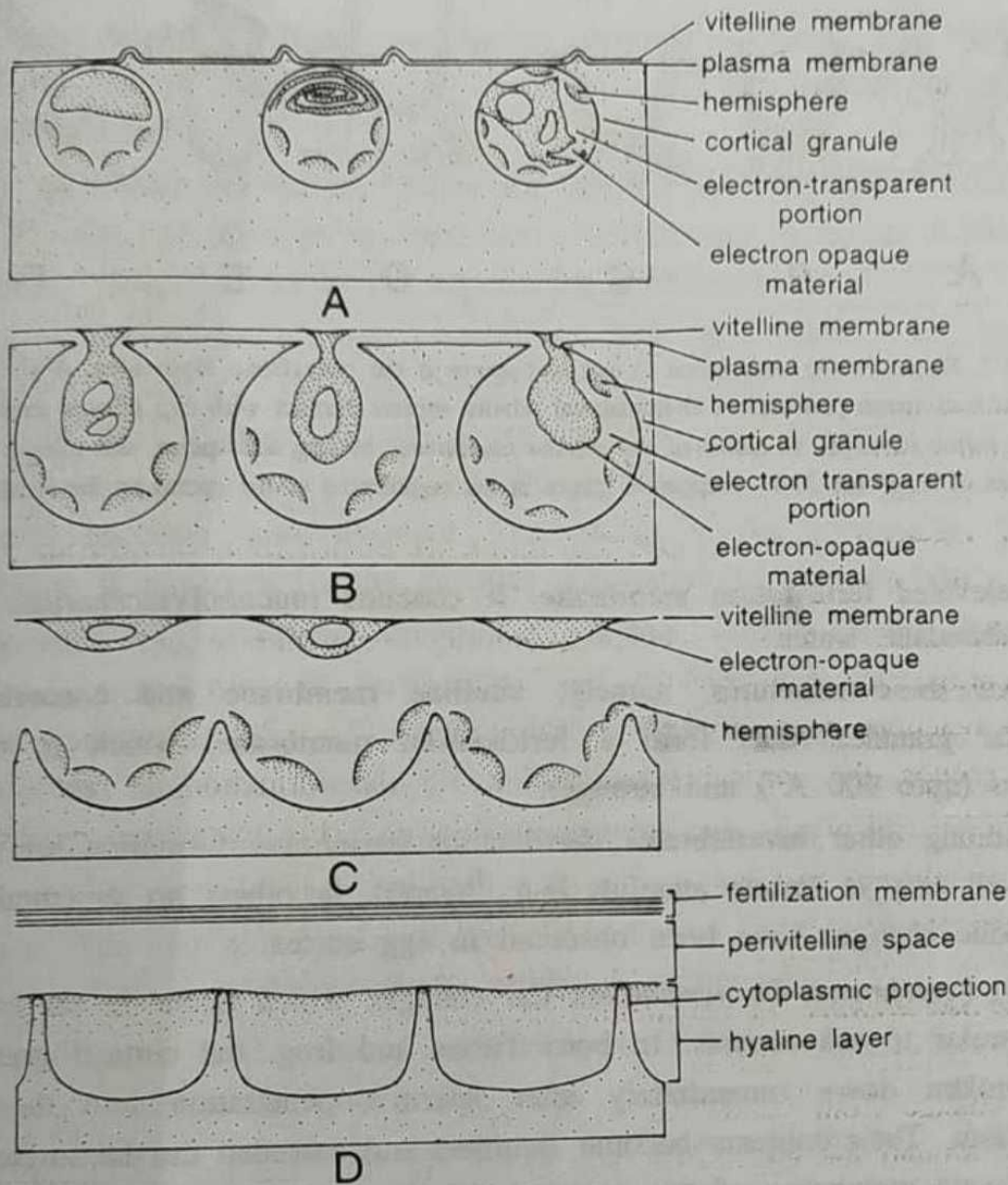


Fig. 4.6. Cortical reaction and fertilization membrane formation in the sea urchin *Clypeaster japonicus*. A—unfertilized egg, B—fusion of egg plasma membrane and membrane of cortical granules, C—adhesion of electron opaque material to the vitelline membrane, D—egg surface after completion of cortical events.

- (1) **Dark, denser, lamellar** and **folded** parts of the granules—these lamellar bodies unfold and fuse with the inner side of the already elevating membrane, the vitelline membrane.
- (2) **Globules**, which fuse together and build up a new surface of the viscous hyaline layer, just at the outer side of the egg plasma membrane. The hyaline layer adheres closely to the surface of the egg and during cleavage, it helps to keep the blastomeres together.
- (3) **The liquified component** of the cortical granules fills the perivitelline space between the new egg surface and now the completed and

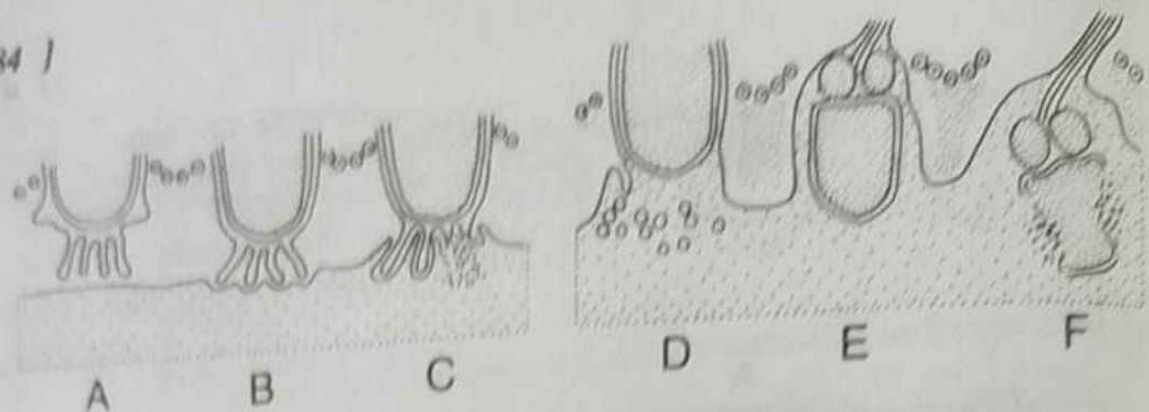


Fig. 4-7. Stages in the association of egg and sperm in the polychaete *Hydroides*. A—acrosomal tubules lead sperm penetration, B—acrosomal tubules initiate contact with egg plasma membrane, which forms microvilli, C—fusion of the plasma membranes of egg and sperm, and release of the contents of acrosome, D-F— successive stages in the engulfment of the sperm by the fertilization cone.

elevated fertilization membrane. It contains mucopolysaccharides and abundant water.

All these structures, namely, vitelline membrane and contents of cortical granules, thus form a fertilization membrane, which is much thicker (upto 900 A°) and stronger.

Among other invertebrates, fertilization membrane formation has been observed only in certain annelids (e.g., *Nereis*). In others no structural but metabolic changes have been observed in egg cortex.

(b) *Vertebrates*. In vertebrates the changes which occur in the cortex are similar to sea urchins. In bony fishes and frog, the cortical granules are broken down immediately after sperm's penetration into the egg cytoplasm. Their contents become liquified and extruded on the surface of the plasma membrane of the egg and fill the perivitelline space occurring in between the chorion and plasma membrane and in between the vitelline membrane and egg plasma membrane in the frog. In both cases, the vitelline membrane or chorion itself does not transform into the fertilization membrane as in sea urchins. The chorion in fishes becomes hardened or "tanned" after fertilization and no new membrane is formed in either of the two animals.

In some mammals (man, rabbit, etc.) the cortical granules burst and release their contents into the perivitelline space created between the egg plasmalemma and the zona pellucida.

In urodele amphibians and some mammals, which lack cortical granules, neither any cortical reaction nor fertilization membrane formation occurs.

3. Metabolic activation. After the sperm penetrates the unfertilized egg a series of diverse cytoplasmic reactions are initiated. Following metabolic changes occur in the egg during fertilization.

(a) **Changes in plasma membrane.** The permeability of plasma membrane increases for the molecules of water and certain other chemicals like ethylene glycol, phosphate, K^+ etc. At fertilization, the electrical potential of plasma membrane becomes more positive and it gradually becomes more negative. The change in the potentiality of the membrane is governed by the unequal distribution of chloride ions.

Besides this, a plasma membrane enzyme **adenyl cyclase** becomes activated at the time of fertilization and it starts the formation of a chemical molecule **3' - 5' cyclic AMP**, which is supposed to activate most of the metabolic reactions in a fertilized egg.

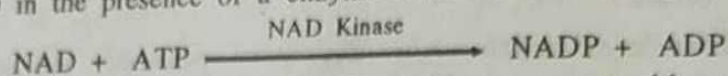
(b) **Ionic changes.** Certain intracellular changes occur in the concentration of cations, especially those of sodium, potassium and calcium. The change in calcium ion concentration in a fertilized egg has great significance in the metabolic activation of the egg.

(c) **Changes in the rate of respiration.** In a fertilized egg, the rate of respiration either increases (e.g. sea urchins) or decreases (e.g. *Chaetopterus* and Molluscs, *Cumingia*) or remains static (e.g. *Bufo* and *Fundulus*). There appears to be a relation between the post-fertilization oxygen consumption and the stage of maturation of the egg at fertilization. Because at the time of fertilization the sea urchin egg has completed maturation, the egg of *Bufo* is at second maturation division stage and the egg of *Chaetopterus* is at the first maturation division stage.

The increased oxygen consumption is related with the oxidation of glycogen and other food stuffs of the egg and synthesis of numerous ATP molecules.

(d) **Co-enzyme changes.** The primary action of the spermatozoon consists of the release or activation of the oxidative enzymes of the egg and that the ensuing increase in oxidation provides the energy necessary for the performance of other changes in the egg and for the development of the egg in general.

In a fertilized egg inter conversion of pyridine coenzyme, NAD into another co-enzyme NADP and also NADPH due to phosphorylation of the NAD in the presence of an enzyme **NAD Kinase** takes place.



There are ample evidences that NAD kinase enzyme although present in the unfertilized egg, is in inactive state. It is activated only at the time of fertilization. The increased NADP and NADPH contents may initiate many synthetic pathways of fertilized egg.

(e) *Changes in the rate of protein synthesis.* The cytoplasm of a mature unfertilized egg contains complete machinery for protein synthesis, such as DNA molecules, tRNA, mRNA, ribosomes and proteolytic enzymes required during protein synthesis, but none or very little protein synthesis occurs because the mRNA of unfertilized egg remains "masked". There are evidences that during later phases of oogenesis some inhibitor or repressor proteins are manufactured in sea urchin's egg which inactivate chromosomal genes, mRNA molecules, ribosomes etc. During fertilization there is an increase in proteolytic activity of the egg immediately following the penetration of spermatozoon which removes these inhibitor proteins from them and unmasks the mRNA and active protein synthesis is started. In the egg of frog, however, the rate of protein synthesis is increased quite early at the stage of ovulation itself.

(f) *Initiation of mitosis.* The initiation of mitosis for cleavage is the most significant aspect of egg activation. The initiation of mitosis occurs because (i) the rate of DNA synthesis increases with great pace immediately after fertilization; (ii) the unfertilized egg cytoplasm although possesses a centriole, yet this centriole is incapable of division and also to form a mitotic spindle. Thus sperm stimulates the first mitotic division (cleavage) of fertilized egg by contributing its centriole to the egg.

In some animals after fertilization, considerable amount of acid is formed during first several months.

Theories of activation

The process of activation of egg by sperm during fertilization is still not very clear. However, various theories have been put forward by different embryologists to explain the mode of activation of egg. Some of the important theories concerning the activation of egg by sperm are as follows :

1. **Bataillon's theory.** Bataillon (1910, 11, 12 and 16) suggested that the exudation or the excretion of substances into the perivitelline space

and elevation of the fertilization membrane activate the egg. He believed that the unfertilized egg was inhibited because of accumulation of metabolic products and the activation or fertilization led to release of these substances to the exterior of the egg.

2. Sensitization to calcium theory. The importance of calcium in egg activation has been emphasised in two independently developed theories, namely "**sensitization to calcium**" theory of Dlacq, Pasteels and Brachet (1936) and the "**Calcium release-protoplasmic clotting**" theory of Heilbrunn (1930-1940). These theories revealed that (i) isotonic calcium chloride solutions or calcium rich solutions are effective activators in many species of animals; (ii) activation of other chemical agents is dependent upon the presence of calcium ions; and (iii) depriving the eggs of calcium will sensitize them to subsequent action of calcium solution.

3. Loeb's theory. Loeb (1964) believed that in normal fertilization the sperm brings in a lytic principle which brings about cortical cytolysis and a second substance called "**corrective factor**" which regulates oxidation. Lovtrup (1974) has supported Loeb's theory of activation. According to him there may be some cytolytic agents which may function as activators by causing a partial dissolution and perforation of plasma membrane opening of cortical granules and subsequent swelling of perivitelline space and further activation of fertilized egg.

4. Lillie's theory. R.S. Lillie (1941) suggested that the cortical changes are the main aspects of activation of egg. The decrease in viscosity permits the interaction of various cytoplasmic substances which normally are kept separated in the unactivated egg. The activating substance 'X', formed in the egg is comparable to the growth hormone. This substance is formed by the union of two substances 'Y' and 'Z' that may be initially present in low concentration in the egg.

Substance 'Y' is considered to be a product of hydrolytic process in the egg and its formation is stimulated by the action of such agents as heat and acids, which can act under anaerobic conditions. Substance 'Z' is considered to be formed by synthetic processes that may be stimulated by agents like hypertonicity acting only in aerobic conditions. Since some substance of each type is present, the threshold concentration of 'X' can be reached by increase in either 'Y' or 'Z'.

5. Repressor theory of activation. Monroy (1965) has given some convincing arguments about activation of egg by sperm in molecular terms. According to him, during maturation of egg, the energy yielding systems

are blocked, so all the reactions requiring a large amount of energy are also inhibited. The accumulation of inhibitory substances during maturation of egg seems to be the most likely explanation and indeed, he recognised one such inhibitor. However, how such inhibitions are produced during the final phase of maturation and how they are inactivated or disposed off by the egg following fertilization still remain to be explained.

Monroy's repressor theory has been supported by many embryologists such as Tomkins, *et. al.* (1969). Metafora, *et. al.*, (1971), D. Epel (1977), Berrill and Karp (1978), and Balinsky (1901). Now there are large number of evidences in favour of the fact that the repressor substances manufactured by the egg in the later phase of maturation inhibit both the metabolic activities residing in the cytoplasm and the genetic activities of the nucleus. The key reaction of fertilization is the removal of the repressors, releasing at the same time the cytoplasmic metabolic activities and the activity of the nuclear genetic system.

Components of spermatozoon in the egg interior

Many variations have been observed in different groups of animals, as to how much part of the spermatozoon is engulfed into the interior of egg during fertilization. In most cases the sperm nucleus, periacrosomal material, proximal centriole and mitochondria make their entry as a rule. The plasma membrane of the sperm becomes one of the entity of plasma membrane of the egg. In mammals, complete structure of spermatozoon (i.e. head, middlepiece and tail) penetrates into the egg cytoplasm. In echinoderms the sperm tail remains exterior to the vitelline membrane while in *Nereis*, only sperm nucleus and proximal centriole enter the egg cytoplasm. There is no definite proof that any constituent of the spermatozoon except for the nucleus and centrosome play any active part in subsequent development. The mitochondria have been observed in some cases to scatter in the cytoplasm of egg, but it is not known how long they maintain their existence there.

In vertebrates, as a rule the egg completes its first meiotic division in the ovary and reaches the metaphase stage of the second meiotic division. At this stage all further progress is arrested, ovulation takes place and the egg may become fertilized. The second polar body gets extruded only if the egg is fertilized by a spermatozoon or activated in some other way. In ascidians, the egg reaches only the metaphase of the first meiotic division

when it becomes ripe and if fertilized, only then the egg completes the first reduction division and carries out the second meiotic division.

V Migration of pronuclei and amphimixis

At the time of penetration of spermatozoon inside the egg cytoplasm, the sperm nucleus remains compact and its mitochondria and centriole remain situated behind it. To perform the act of amphimixis, the sperm nucleus has to undergo two activities (i) it has to become pronucleus, and (ii) it has to migrate from the site of amphimixis. As the sperm nucleus moves inwards from the site of fertilization cone, it soon rotates through an angle of 180° , so that its mitochondria and centriole assume the leading position. Besides this rotation, the sperm nucleus starts swelling and its chromatin, which is very closely packed, becomes finely granular. It ultimately becomes vesicular and has an appearance like the interphase nucleus and is called **male pronucleus**.

At the same time, the sperm aster forms around the proximal centriole of the sperm in the egg cytoplasm. As the male pronucleus develops and migrates towards the site of amphimixis, the sperm aster seems to lead it. The site of amphimixis lies either near the centre of microlecithal and mesolecithal eggs or in the centre of the active cytoplasm at the animal pole of macrolecithal and telolecithal eggs. As the sperm pronucleus and centriole move inward, it may be accompanied by some cortical and subcortical cytoplasm. If the latter is heavily pigmented, as in amphibian eggs, the trajectory of the sperm pronucleus may be marked by pigmented granules trailing along its path. This is called **penetration path**. This movement of the sperm appears to be directed and some investigators feel that it is due to a chemotaxic effect of chemicals liberated by the **female pronucleus**. During this movement toward the female pronucleus, the sperm may have to deviate from its penetration path. If it does, the new pathway taken is referred to as **copulation path**.

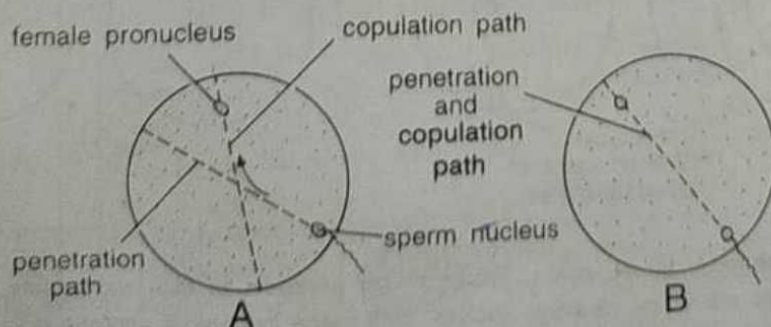


Fig. 4-8. Possible sperm paths during fertilization.

In some cases the sperm need not alter its direction. In these cases the penetration and copulation paths would be identical. The point of entrance, penetration path and copulation path are all believed to be responsible for establishing the primary plane of bilateral symmetry in the embryo.

Before amphimixis the nucleus of the egg also undergoes certain changes like the sperm nucleus. After the completion of the second meiotic division, the haploid nucleus of the egg is located near the surface of the egg in the form of several vesicles, known as **karyomeres**. In a fertilizing egg, these karyomeres fuse together to form a **female pronucleus** which swells, increase in volume and becomes vesicular. It also migrates towards the site of **amphimixis**.

Amphimixis

The fusion of male and female pronuclei is called as **amphimixis**. The actual fusion of pronuclei may differ in different animals.

- (1) In sea urchins and vertebrates, the nuclear membranes of both pronuclei are broken down at the point of contact and their contents unite in one mass surrounded by a common nuclear membrane. At the approach of first cleavage of fertilized egg, the nuclear membrane dissolves, chromosomes of maternal and paternal origin become arranged on the equator of the achromatic spindle.
- (2) In *Ascaris*, some molluscs and annelids, the male and female pronuclei don't fuse but the nuclear membranes in both dissolve and the chromosomes become released. In the meantime, the centrosome of the spermatozoon has divided into two and the centrosomes derived from male and female pronuclei become attached to the

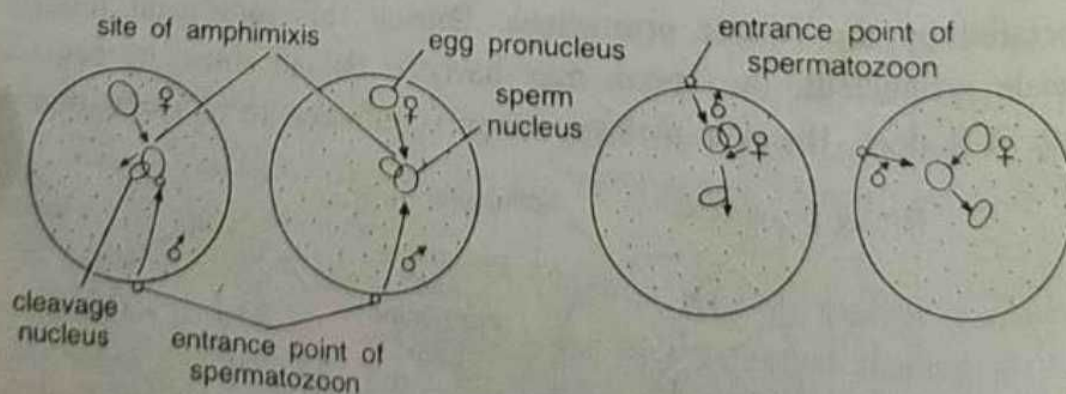


Fig. 4-9. Amphimixis in sea-urchin. Tracks of the pronuclei in their movements toward each other and to the site of the cleavage nucleus, with sperm entering at opposite side of egg from the site of egg nucleus and from the same side, respectively.

spindle. Only after the completion of the first division of the fertilized egg, the paternal and maternal chromosomes become enclosed by common nuclear membrane to form the nuclei of two blastomeres into which the egg has been divided.

In both these types (1 and 2), the chromosomes of maternal and paternal set retain, of course, their individuality.

- (3) In the Copepod *Cyclops*, the paternal and maternal nuclear components remain separate for some time even after cleavage has started, so that each blastomere has a double nucleus consisting of two parts lying side by side and each surrounded by its own nuclear membrane.

Post-fertilization changes in the egg cytoplasm

The penetration of spermatozoa into the egg causes far-reaching displacements of the cytoplasmic constituents. As a result, the distribution of various cytoplasmic substances and inclusions in a fertilized egg may be very considerably different from that in the unfertilized egg and even quantitatively new areas may appear. As a result of the extrusion of cortical granules, a large part of the original outer egg cell surface is replaced by the inner surface which surrounds the cortical granules and now are everted on the exterior. Most spectacular post-fertilization displacements in the egg cytoplasm have been observed in ascidian, *Styela partita* and in frog. In both these animals, a bilateral symmetry established in the cytoplasm of fertilized eggs.

Displacements of cytoplasmic substances in the egg of ascidian *Styela partita* are as under :

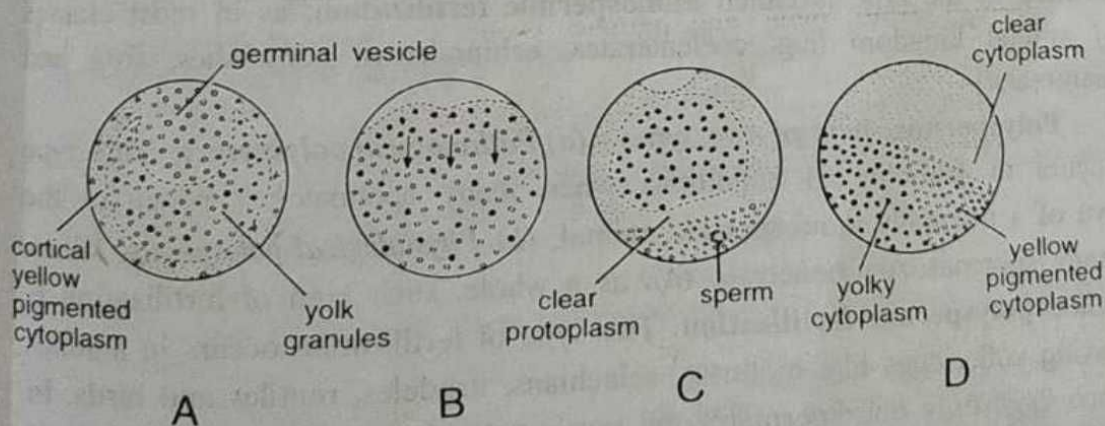


Fig. 4-10. Displacements of cytoplasmic substances in the egg of the ascidian, *Styela partita* following fertilization. A—Ripe unfertilized egg ; B—Egg soon after the entry of spermatozoon; C-D—Male pronucleus moves to meet female pronucleus and the formation of cytoplasmic territories.

The mature egg is covered by a layer of cortical cytoplasm containing yellow granules. The moment spermatozoon enters the egg near the vegetal pole, the yellow cortical cytoplasm falls into violent commotion.

The yellow cytoplasm begins to stream down along the surface of the egg toward vegetal pole, and accumulated as a cap. As the male pronucleus penetrates deeper into the cytoplasm and moves toward female pronucleus, the yellow cytoplasm reverses its movements and streams upward only on the side where the spermatozoon entered the egg. Just before the first cleavage, the yellow cytoplasm forms a crescentic area (**mesodermal crescent**) just below the equator of the egg. Simultaneously, a crescent of light gray cytoplasm (**notochordal crescent**) appears subequatorially on the opposite side of the egg. Thus, in a fertilized egg, four different kinds of cytoplasmic regions are now present— (i) The yellow cytoplasm on one side, and (ii) the light cytoplasm on the other side. These two together form a belt surrounding the egg just below the equator. Below this zone toward the vegetal pole, (iii) the cytoplasm in slaty grey colour contains abundant yolk granules and in the subcortical layer, there are a large number of mitochondria. But the cytoplasm in (iv) the animal hemisphere contains less yolk and a few mitochondria and appears more transparent. Thus, the cytoplasmic displacements following fertilization not only bring some kind of cytoplasm to more restricted areas, but also give the egg a distinct bilateral structure.

Monospermic and polyspermic fertilization

Monospermic. When only one spermatozoon penetrates into the egg to fertilize it, the type is called **monospermic fertilization**, as in most classes of animal kingdom (e.g. coelenterates, echinoderms, bonyfishes, frog and mammals.)

Polyspermic. It is of two types—(a) **Pathological polyspermy**. This type occurs in pathological conditions when many spermatozoa penetrate the ova of a normally monospermic animal. (b) **Physiological polyspermy**. When many spermatozoa penetrate ova as a whole, such kind of fertilization is called **polyspermic fertilization**. This type of fertilization occurs in animals having yolky eggs like molluscs, selachians, urodeles, reptiles and birds. In such eggs only one spermatozoon participates fully in the development of the embryo, the rest degenerate sooner or later. This type of polyspermy, because it has some physiological significance, is called **physiological polyspermy**.

Significance of fertilization

The process of fertilization has following significances :

- (1) The fertilization ensures the usual specific diploidy of the organisms by the fusion of the male and female pronuclei.
- (2) It introduces genetic variations in the species.
- (3) It activates the egg to start cleavage.

IMPORTANT QUESTIONS**» Long answer questions**

1. Describe the process of fertilization. What is its significance?
2. Discuss how the ovum is activated during the process of fertilization.
3. Write short notes on— (i) Acrosome reaction and penetration, (ii) Activation of ovum, (iii) Cortical reaction and fertilization membrane formation, (iv) Amphimixis.

» Short answer type questions

1. Define fertilization?
2. What are the basic requirements for fertilization?
3. What is fertilizing - antifertilizin reaction?
4. How does penetration of sperm into ovum take place?
5. What is the function of acrosome in sperm?
6. What is cortical reaction?
7. How is fertilization membrane formed?
8. How is polyspermy prevented?
9. Give any four metabolic changes in the ovum followed by fertilization.
10. What is the significance of fertilization?

» Multiple choice questions

1. One of the fundamental requirement for the encounter of spermatozoa and egg during fertilization is
 - (a) fluid medium
 - (b) dry medium
 - (c) jelly-like medium
 - (d) none
2. Chemical nature of fertilizing is
 - (a) protein
 - (b) lipid
 - (c) glycoprotein
 - (d) carbohydrate
3. In case of eggs with very thick and resistant membranes, the sperm reach the eggs and penetrate through a special canal known as
 - (a) fertilization canal
 - (b) micropyle
 - (c) fertilization cone
 - (d) fertilization point
4. Sperm head penetrates the corona radiata with the help of a substance by acrosome known as
 - (a) lyase
 - (b) glucuronidase
 - (c) hyaluronidase
 - (d) urease
5. The cytoplasm of egg bulges forward at the point of contact of acrosome tubule and egg plasma membrane. This is called as
 - (a) micropyle
 - (b) fertilization cone
 - (c) fertilization point
 - (d) penetration point