Chapter 3
Fertilization

Gametogenesis produces specialized cells designed to unite and form a **zygote**. Fertilization is the actual union of the male and female gametes. This includes the penetration of the ovum by the spermatozoon and the pooling of the nuclei. This mixing of the two chromosome pools is called **amphimixis**. At fertilization the chromosome number becomes diploid again. Another aspect of fertilization is the **activation** of the egg. This refers to the many changes which occur in the ovum as a result of sperm penetration. Activation is divided into early responses, which function to prevent polyspermy, and late responses which include metabolic changes leading to cell division. The most fertilization research has been done with sea urchins and mammals.

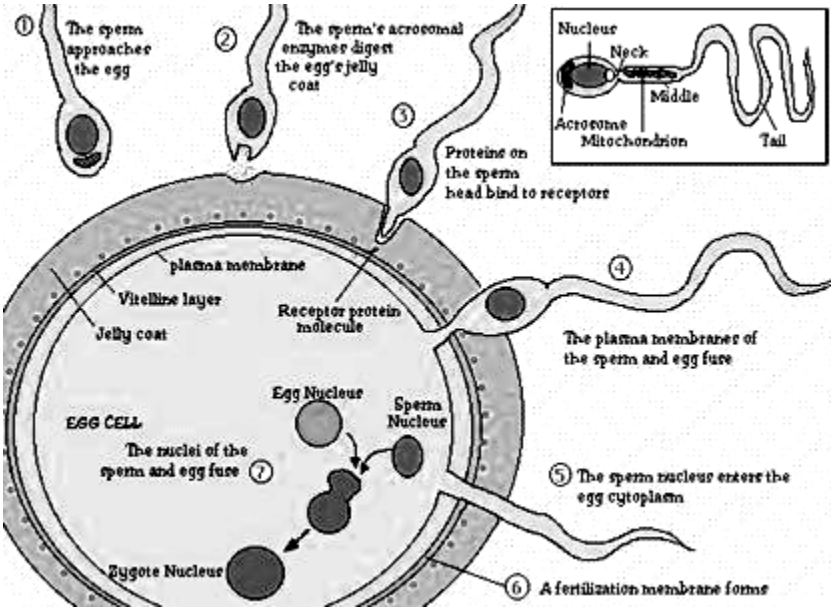


Fig.3.1. Events of Fertilization

3.1. Location of Ovum by the Spermatozoon

When sperm are released into the genital duct of the female or into water, during mating or **spawning,** the **spermatozoa** must locate the egg. In most mammalian species the sperm must undergo **capacitation**. The capacitated sperm are more active and the flagellum beats faster. This process typically occurs after several hours of exposure to fluid from the uterus or oviduct. *In vitro* fertilization requires capacitated sperm. Similar behaviour has been observed in some invertebrates, as well. Studies have shown that the sperm in many species display **chemotaxis** toward a chemical released by the egg. In mammals the sperm are deposited in the vagina or uterus. In humans 100-500 million sperm are deposited, but only a few hundred reach the fertilization site in the upper oviduct. Uterine and vaginal contractions help move the sperm, as well as chemotaxis.

The sperm then collect at a site just below the fertilization site. There is evidence that those mammalian sperm *(in their optimal metabolic state)* are selectively activated by a

chemical from the ruptured follicle.
Some animal eggs rely heavily on **chemotaxis** to orient spermatozoa, because the sperm does not have free access to the ovum, but must enter at a specialized opening. *Campanularia* is a hydroid animal in which the eggs are surrounded by theca; thus, the sperm must enter through an opening in the theca. In some fish (Teleosts) the eggs are covered with a tough chorion. The chorion has canals through it called **micropyle**. The sperm must find its way through the micropyle to fertilize the egg. These chemical attractants have been shown to species-specific. In sea urchins a species-specific attractant, called **resact**, has been identified as a peptide, made of 14 amino acids.

3.2. Sperm Penetration

(a) Summary of Fertilization Chronology

1. Sperm **attach** to the **Zona Pellucida (ZP)**, which is relatively **non-specific.**
2. Sperm **bind** to ZP by a **species-specific** reaction between the *ZP sperm receptors* of the egg & the *complimentary egg binding proteins* of the sperm.
3. The Acrosomal Reaction of bound sperm allows sperm penetration of the ZP and sperm fusion with plasmalemma, creating a fast block to prevent polyspermy.
4. The egg is activated, causing the cortical reaction, which induces the zona reaction.

(b) Sperm Receptors of the ZP

The ZP contains species-specific sperm receptors, which are deactivated after fertilization, thus preventing polyspermy. **Mouse sperm receptors** are designated as **ZP1, ZP2 and ZP3**. ZP3 is a glycoprotein, with a MW of 83000 daltons; one of its oligosaccharides binds, in a species-specific manner, to the sperm. ZP2 is responsible for maintenance of sperm binding to the egg. In the **Sea Urchin**, there are sperm receptors on the vitelline envelope (VE). The cortical reaction converts the VE to a fertilization envelope, to which sperm cannot bind.

(c) Egg-binding Proteins of the Sperm

Sea urchin eggs contain specific receptors for the sperm protein, **bindin.** Bindin is a major component in the **acrosome**. It binds to the **vitelline membrane** of the egg and is species-specific. In mammals, molecules like bindin have not been found, but in mice several different kinds of proteins are being considered as candidates for a role as an egg-binding protein, similar to bindin in the Sea Urchin. These are: lectins, glycosyl transferases, proteases and glycosidases. In mammals, an influx of Ca^{2+} into the sperm initiates the **acrosome reaction**. This is mediated by the polypeptide component of ZP3. The oligosaccharide component of ZP3 is involved in specific binding to sperm receptors.

(d) Sperm Penetration of the Egg Membranes

Recall that eggs are usually surrounded by membranes and also may be surrounded by follicle cells (as in mammals). Sperm penetrate these barriers by releasing chemical substances called sperm **lysins**, which are found in the acrosome. The lysin chemical structure varies from one species to the next. In **mammals** the enzyme **hyaluronidase** is a lysin; it specifically dissolves the **mucopolysaccharide, hyaluronic acid**. A second enzyme is the **corona-penetrating enzyme**. Once sperm penetrate the **zona pellucida,** they contact and fuse to the egg plasmalemma. Only acrosome-reacted sperm can bind to egg plasmalemma. This is not as species-specific as the binding to the zona pellucida. In

the mouse the entire sperm head moves through the zona pellucida.
In the **invertebrates**, such as the **sea urchin,** as the sperm approaches the egg, the acrosome undergoes a major change, which is initiated by some chemical from the egg. A long acrosomal filament grows out from the acrosome. This acrosomal filament dissolves its way through the membranes of the egg and makes contact with the egg plasmalemma. A proteolytic enzyme, **acrosin**, is involved in this process.
After the sperm are bound, the egg plasmalemma surrounds the sperm with thousands of microvilli, forming a **fertilization cone**, which engulfs the sperm and then retracts, pulling the sperm into the egg. The cortex contains contractile proteins, actin and myosin. In sea urchins, bundles of actin filaments extend into the cortex from the microvilli in the area where the acrosomal filament has made contact.

(e) Polyspermy

Polyspermy is when more than one spermatozoon enters the egg. In some animals (mollusks, urodeles, reptiles and birds) polyspermy is the general rule. More than one spermatozoon enters the egg, but only one sperm nucleus combines with egg nucleus, while the others die. This is called **physiological polyspermy**.
Normally, in most animals, the egg is fertilized by only one spermatozoon. This is **monospermic fertilization**. In these animals, if more than one sperm fertilizes the egg, the embryo is not viable and dies. This is **pathological polyspermy**.

(f) Prevention of Polyspermy

In the 1950's, **Lord Rothschild** & **Michael Swann** (of England) suggested that two separate barriers to polyspermy exist. The first is a fast, incomplete block, which occurs within the first few seconds of sperm-egg contact; this is followed by a second, slower, more complete block. Studying the nature of the fast change, **Laurinda Jaffe** (at UCLA) inserted microelectrodes into sea urchin eggs. About one second after the attachment of the sperm there is a flow of Na^+ into the cell causing a brief voltage shift, resembling that of a nerve impulse. This voltage shift is apparently responsible for keeping supernumerary sperm from entering the egg. Further, if the voltage is increased artificially across the membrane of unfertilized eggs, sperm are unable to fertilize the eggs. Other fast block systems have since been identified in anurans, starfish and echiuran worms but there is no electrical block in mammals. Investigations of the slower block to polyspermy have focused on the **cortical reaction** which blocks in three ways:

1. Elevation of the fertilization envelope
2. Inactivation of sperm receptors
3. Hardening of the fertilization envelope

Cortical granules contain a mixture of enzymes, structural proteins and colloidal materials (mucopolysaccharides). One of the enzymes breaks attachments between the plasma membrane and vitelline envelope, allowing the two membranes to separate. The mucopolysaccharides are colloids, which are released into the perivitelline space. Therefore, H_2O osmoses into the perivitelline space lifting the vitelline envelope away from the surface of the egg. Another enzyme, specifically alters the sperm-receptor proteins on the vitelline envelope, so that supernumerary sperm already bound are detached and additional sperm cannot bind. Within a minute of sperm-egg contact structural proteins from the cortical granules associate with the vitelline envelope, transforming it into a protective envelope, the fertilization envelope. In some mammals,

release of cortical granule contents results in the modification of the zona pellucida. The zona hardens and inactivates sperm receptors; this is called the zona reaction.

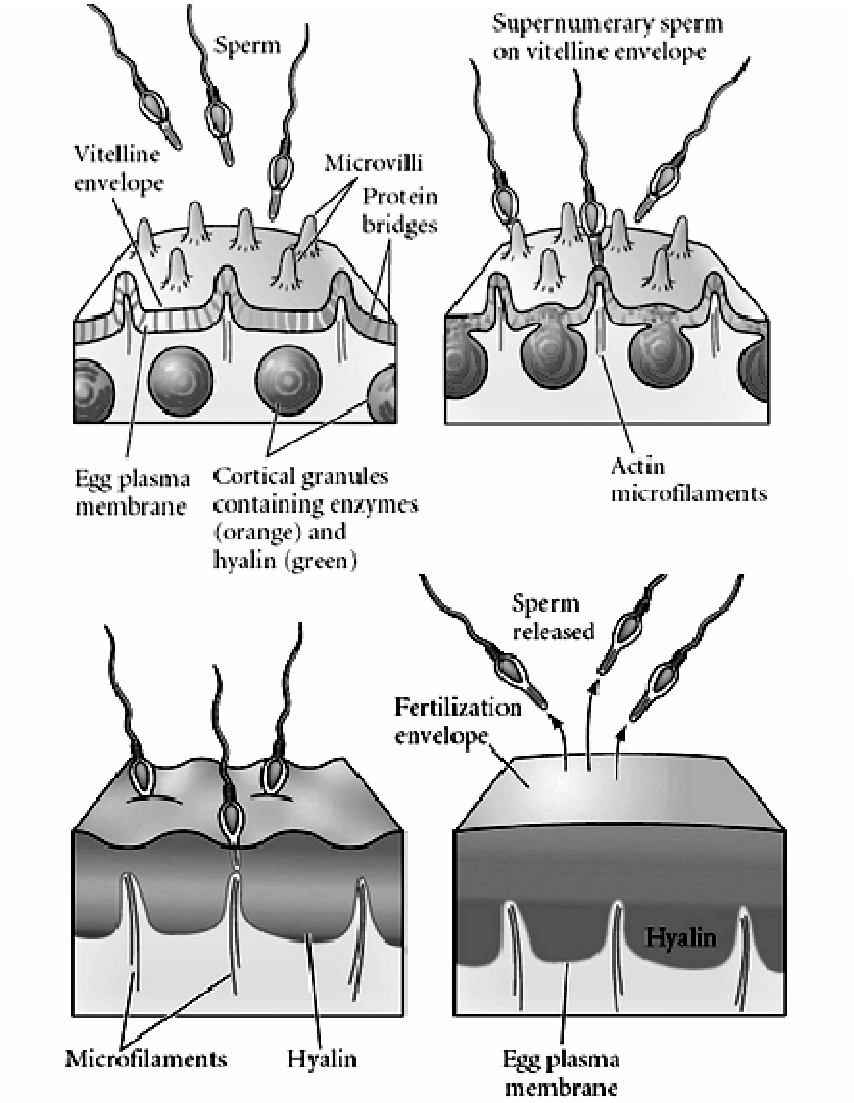


Fig.3.2. The cortical reaction is responsible for the slow block to polyspermy

3.3. Activation

Up until fertilization the egg was in a resting state; upon fertilization the egg sets its development machinery into motion. That is, the egg undergoes a number of changes which readies it for development.

1. Before fertilization, the egg exists in a metabolically repressed state; respiration, transport of substances into and out of the cell, protein and RNA synthesis are all reduced and DNA synthesis is shut down.

2. Upon fertilization, there is a general activation of the dormant metabolism of the egg. The metabolic changes are precipitated by the sperm contacting an egg membrane, triggering the activation a receptor.

3. Many of the activation events are caused by **calcium ion transients**, momentary spikes in cytoplasmic levels of Ca^{2+}, which is released from intracellular stores in

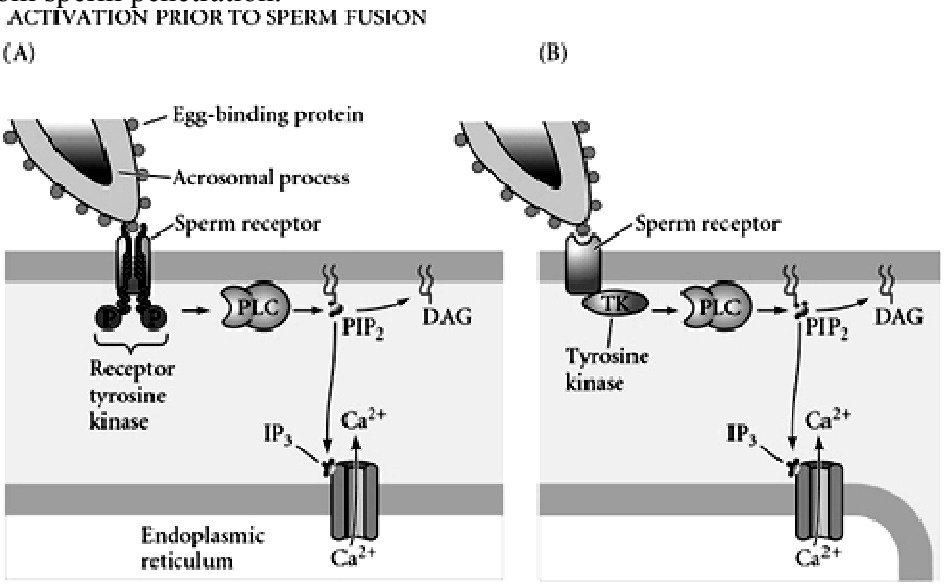
response to common secondary messenger molecules (*viz.* inositol triphosphate, IP_3).

B. Some of the earlier changes are a number of changes that occur at the outer cytoplasm (cortex) of the egg. These are collectively called the **cortical reaction** and vary widely from one species to the next.

In **Sea Urchins**, beginning at the point of sperm-egg fusion, the cortical granules swell and explode releasing their contents into the **perivitelline space** (between the plasma membrane and the vitelline envelope). The VE separates from the plasma membrane and is now called the **fertilization envelope**. The material released from the **cortical granules** reinforces the fertilization envelope, expands the perivitelline space and forms a viscous **hyaline layer** at the surface of the egg which helps hold the dividing cells together during cleavage. The ruptured plasmalemma restores itself.

The cortical reaction in sea urchins differs from cortical reactions in other animals. Frogs and bony fish already have membranes equivalent to a fertilization envelope prior to fertilization. In frogs this is the vitelline membrane and in fish it is the chorion. At fertilization the cortical granules rupture and the perivitelline space expands. In **Lamellibranch mollusks** (largest subclass of Bivalvia *(Pelecypoda)*) the cortical granules do not change at fertilization, but just gradually breakdown; this process starts before fertilization.

Some animals (some mammals, urodeles, some insects *etc*.) do not have cortical granules and there is no observable cortical reaction. Activation causes **movements of the cytoplasmic constituents**. A good example of this is the frog egg. The cortical cytoplasm at the animal pole is dark with pigment granules. At the vegetal pole, the cortical cytoplasm is clear, with no pigment granules. As a result of sperm penetration the cortical cytoplasm rotates relative to the internal cytoplasm. The cortical cytoplasm moves upward (toward the animal pole) on the future dorsal side of the egg and moves downward (toward the vegetal pole) on the future ventral side of the egg. As the dark cortical cytoplasm moves up from the equator on the dorsal side of the egg, it reveals a marginal ring of lighter pigmented cytoplasm below the cortical layer. This area becomes the **gray crescent**, which marks the future symmetry of the zygote; the first cleavage furrow is always perpendicular to it. In the frog the gray crescent will appear on the opposite side from sperm penetration.



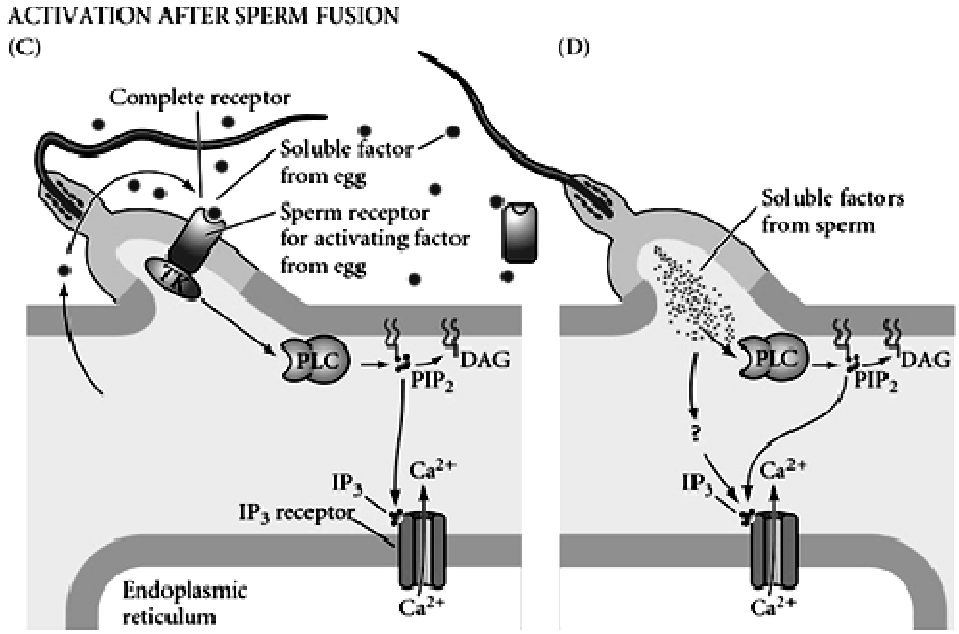


Fig.3.3 Alternative activation pathways

3.4. Fusion of Nuclei

In some animals, like mammals, the entire spermatozoon enters the egg and the tail breaks down inside egg. In others, like **echinoderms**, the principle piece and end piece of the tail are left outside of the egg. In yet others, like the **polycheate worm**, *Nereis*, only the head enters the egg. In those that middle piece enters, the mitochondria disperse into egg cytoplasm and their fate is unknown. The sperm nucleus may have to wait for the egg to complete its **meiotic divisions**. In the vertebrates the usual rule is that the egg has completed the first meiotic (reduction) division and has reached metaphase of the second division; it will complete the 2nd meiotic division only if fertilized. In some animals, like sea urchins, the egg has completed meiosis before it is fertilized. Other animal eggs may arrest meiosis at (or even before) the first meiotic division; after fertilization meiosis will be completed.

After entry, the sperm nucleus is referred to as the **male pronucleus**. The male pronucleus swells and the chromatin becomes more dispersed. After meiosis the egg nucleus is called the **female pronucleus**. The female and male pronuclei move toward one another and then fuse, allowing **amphimixis** to take place. The pronuclei are moved by spindle fibers from the MicroTubule Organizing Center (MTOC). The diploid zygotic nucleus is called the **synkaryon**. In some animal species fusion does not occur, but rather, the pronuclear envelopes break down and the chromosomes then line up on the equator. This type of synkaryon formation is called **approximation**.

3.5. Gene Expression at Fertilization

(a) Protein Synthesis

Protein synthesis is very low in the unfertilized egg, but increases with fertilization. Since both, mRNA and ribosomes, are present in unfertilized eggs, it is probable that they are masked (*viz.* temporarily deactivated) by a protein. mRNA is normally complexed to proteins in cells, forming mRNP particles. Isolated ribosomes from unfertilized eggs are not capable of protein synthesis. But, if they are treated with **trypsin** (a proteolytic

enzyme) they become capable of synthesizing proteins. Therefore, activation may involve production of proteolytic enzymes which remove proteins from the mRNA and/or ribosomes, thereby unmasking them.
Other alternatives for this delayed increase in protein synthesis include: changing the structure of mRNA to make it available, or to sequester the mRNA until the egg is fertilized. In the **ooplasm** the mRNA is apparently bound to the cytoskeleton. During cleavage, cells are specialized for rapid cell division & utilization of stored energy (in the form of yolk). They require special structural proteins: proteins of mitotic spindles, membrane systems (plasmalemma, nuclear envelope, endoplasmic reticulum), major enzymes (for DNA synthesis, energy utilization etc). Some of these proteins are in the ooplasm at fertilization but are not in sufficient quantity to sustain the embryo through cleavage.
In non-mammalian species the egg cytoplasm is pre-programmed to direct protein synthesis through cleavage and the formation of a blastula. Therefore, oogenic mRNA **is** found in the ooplasm and **is** translated after fertilization. The high rate of protein synthesis is triggered and maintained by the **alkalinization** of the cytoplasm during activation.
The increase in protein synthesis involves the mobilization of previously masked mRNA and an increased efficiency of the ribosomes. The rate of chain elongation of polypeptides also increases, independent of pH changes; Ca^{2+} release at fertilization may be responsible for this increase.

(b) RNA Synthesis

In **sea urchins**, some mRNA is synthesized exclusively during oogenesis, some exclusively during cleavage and some during both. There is some rRNA synthesis during cleavage, but at a much reduced rate. The vast majority of rRNA genes remain inactive during cleavage. Most of the RNA in the unfertilized sea urchin egg is rRNA.
In **Amphibians**, most of the RNA synthesized in early cleavage is pre-mRNA. The small amount of mRNA that is synthesized decays rapidly. There is no tRNA nor rRNA synthesis during cleavage.
All organisms eventually switch from the translation of oogenic mRNA to zygotic mRNA. As embryonic development proceeds there is a shift of control from the maternal genome to the zygotic genome. If cleavage-stage **mammalian** embryos are exposed to **α amanitin** (*a more specific transcription-inhibitor in mammals than **actinomycin D***) then protein synthesis drops abruptly by 24 hours and development stops. Oogenic RNA will support development until the 2-cell stage in mice & 8-cell in rabbits.

3.6. Parthenogenesis

Parthenogenesis is the development of embryos from unfertilized eggs. Parthenogenesis occurs in nature in a variety of animals. Examples include:

1. **Aphids**, **phyllopod crustaceans** and in some **rotifers** (phylum Rotifera); rotifers develop without fertilization at certain times of the year.
2. **Bees** & **wasps** produce females with fertilized eggs and unfertilized eggs develop into males.
3. In one subspecies of *Drosophila* and one of lizards, no males exist.
4. Sometimes **turkey eggs** develop parthenogenetically into adult turkeys.
5. Humans? A study in England could find none. Maybe Jesus Christ for Christains

Artificial parthenogenesis can be induced in **sea urchins** by a long list of chemicals, temperature shocks, electrical shocks, ultraviolet light, shaking and pricking the egg with a needle. All these agents cause a certain amount of sublethal damage which causes damage to the cortex of the egg. Sperm also induce changes to the cortex and this stimulus initiates activation. In frogs, parthenogenesis can be artificially induced by some of the same methods used for sea urchins. With most stimuli the embryos do not progress beyond the cleavage stage.

Pricking frog eggs with a fine glass needle is more efficient to a limited extent. If the needle is contaminated with blood or fragments of cells or if these contaminants are injected into frog eggs with a **pasteur pipette** development of the eggs is improved and some eggs even develop fully.

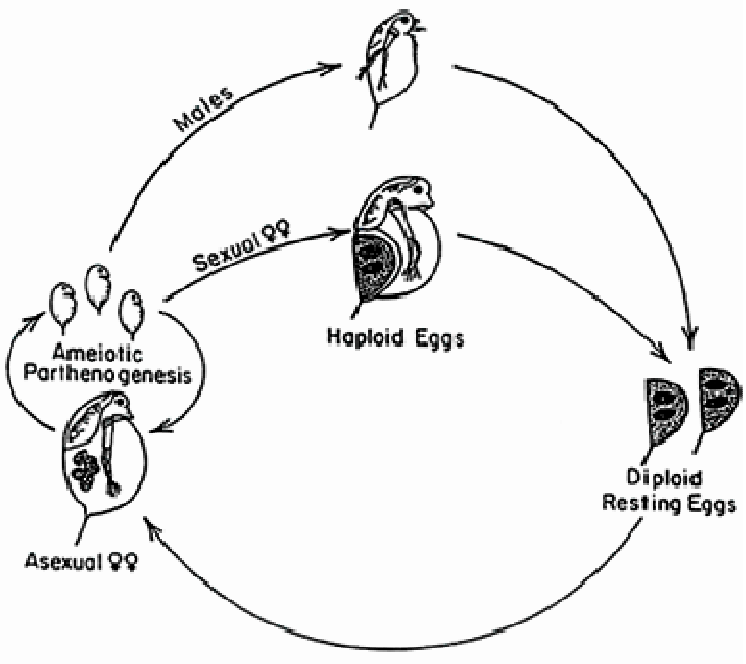


Fig.3.4. Cycle of Parthenogenesis in ***Daphnia***