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Teacher: Praveen Deepak

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ANIMAL FERTILIZATION

Fertilization is a biological process by which a male haploid reproductive cell and a female haploid reproductive cell interact and unite to form a diploid zyogote from which an organism is developed. In other words, it 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. Thus, it indicates that it involves the interaction of two types of cells, one is a sperm and other is an egg or ovum. Sperm or spermatozoa is produced by spermatogenesis as eight round spermatids from single male primordial germ cell which later on transforms into distinct cellular architecture (Figure 1). In the process of metamorphosis of spermatids, spermatid increases in size; centriole divides into two; distal centriole forms the main axis of the tail; the axial filament is surrounded by a fibre coat; mitochondria form a spiral sheath; Golgi complex form acroblast and give rise to acrosome. Therefore, the mature spermatozoa can be visualized into three distinct zones; head, trunk and tail. With the help of tail and mitochondrial in a spiral sheath, it can move actively in fluid.

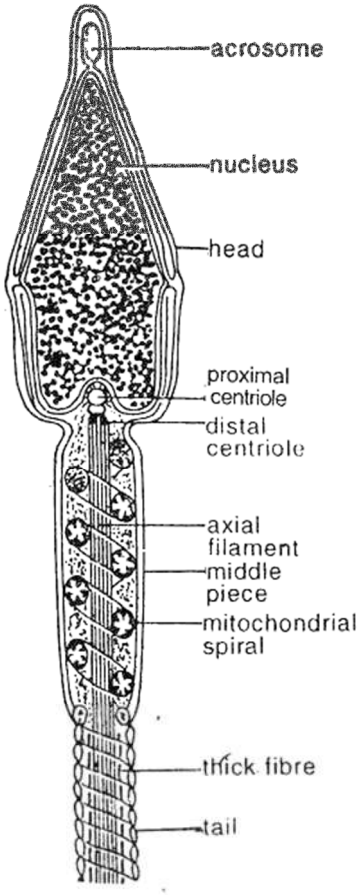

Figure 1. A typical human spermatozoa

- — Head is constituted by an anterior acrosome which is also known as galea capitis and a nucleus. Galea capitis or acrosome is a double-walled, granulated sac (due to the presence of enzymes), convex anteriorly and flat posteriorly. The enzymes, such as hyaluronidase and acrosin present on the acrosome are for dissolving the zona pellucida (egg membrane) of the ovum to facilitate entry of the spermatozoa. The nucleus is a dense structure, rich in DNA, narrower anteriorly and bears a depression at the middle of the broad posterior end.
- — Middle piece connects the head with the tail piece. The proximal centriole of the anterior ends fits into the depression of the head and the distal centriole is attached with the nine axial filaments. The middle piece contains mitochondria spirally arranged around the axial filaments and provides energy for locomotion.
- — Tail portion is clearly divisible into two parts: a broad anterior main piece, and a narrow terminal end piece. The main piece of the tail consists of a spiral mitochondrial sheath surrounding a group of eleven fibrils. The fibrils are centrally placed and the rest form a ring around them. Contraction and relaxation of peripheral fibrils help the spermatozoa to move forward. The enzyme adenosine triphosphatase (ATPase) is present in the whole length of main piece. Terminal end piece is basically a flagellar structure which is slender, naked and resembles as that of cilia.

Correspondingly, ovum or oocyte is produced by oogenesis. In this process, an ovum is produced from single female primordial germ cell through unequal cytoplasmic division. One cell with haploid number of chromosome and with no cytoplasm has no role and is ultimately eliminated.

The oocyte or ovum is a large, round structure (in human, it measure 117 to 142 µm in diameter; Figure 2). The cytoplasm of the ovum is termed **yolk** or **ooplasm**, the nucleus as the **germinal vesicle** and the nucleolus as **germinal spot**. The yolk is distinctly divided into two – a cytoplasm that is simiar to that of other cells, and **deutoplasm** or **nutritive yolk** which is rich in fatty droplets containing lecithin, a phospholipid. The distribution of deutoplasm in the ovum differs in different animals. During oogenesis mitochondria increase in numbers and become uniformly distributed in the cytoplasm. The Golgi apparatus spreads out, being restricted usually near the periphery. The amount of yolk in the ovum varies in different groups of animals. The mature ovum is surrounded by two membranes, the plasma membrane (egg membrane) and the vitelline membrane. The vitelline membrane is thin and surrounds the plasma membrane. In addition to these primary membranes, secondary membrane secreted by the ovary and tertiary membrane secreted by the glands in the oviduct may surround the egg. In branchiostoma, only the plasma membrane and vitelline membrane are present. In frogs and toads, a sphere of jelly around the egg is enclosed in a thin membrane. In birds, a two-layered shell membrane and a calcareous shell are secreted by the oviduct and, in mammals, the additional layers - zona pellucida and chorona radiata can be said as primary and secondary membrane, respectively (Figure 2).

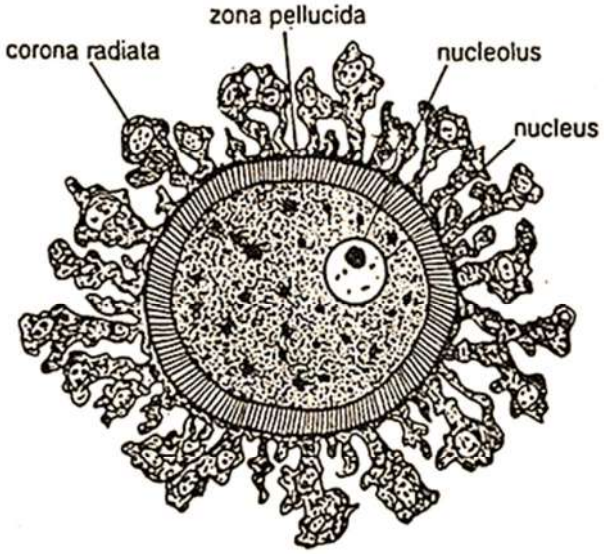

Figure 2. A typical human oocyte

The ova (plural of ovum) or eggs are variously classified on the basis of positions, amount of deutoplasm, polarity, etc. The eggs are of following kinds on the basis of portions and amount of deutoplasm (Figure 3):

1. **Homolecithal or Isolecithal eggs:** In this type of eggs, the mount of yolk is small and present chiefly in droplets and minute spherules, uniformly distributed in the cytoplasm. Eutherian mammals have homolecithal eggs.
2. **Microlecithal eggs:** In this type of eggs, the amount of yolk is small. This type of eggs is found in cnidarians or coelenterates.
3. **Megalecithal eggs:** In this type of eggs, the amount of yolk is large. This type of eggs is found in some invertebrates, reptiles, birds and prototherian mammals.
4. **Centrolecithal eggs:** In this type of eggs, the yolk is present around the nucleus in the form of a large sphere. This type of eggs is found in arthropod eggs.
5. **Telolecithal eggs:** In this type of eggs, the yolk occupies almost the entire egg except a minute area in the animal pole. In chordate, this type of eggs are varied in various animals;

    — In branchiostoma, the yolk is concentrated at one pole, the region of future endoderm cells.

— In cyclostomes and amphibians, the quite high amount of yolk is concentrated in the vegetal pole.
— In reptiles and birds, the amount of yolk is large and occupies almost the entire egg and the active cytoplasm. The egg nucleus forms a small cap on the yolk in the animal pole.

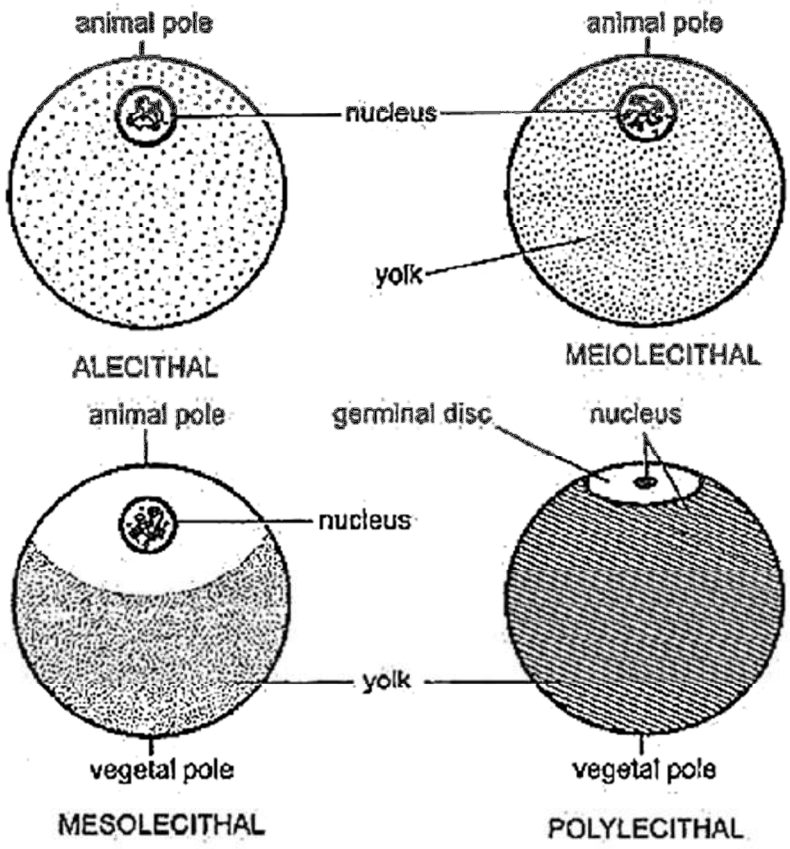

Figure 3. Types of egg cells in different animal

Fusion of these matured and fully differentiated reproductive cell nuclei i.e. complete fusions of spermatozoa and ovum is called as fertilization. Here, chromosomes are not fused but makes a complete set of diploid chromosomes. This union of chromosomes is known as **karyogamy** and that of the cytoplasm is **plasmagamy**. However, in most of cases, male spermatozoa have literally no cytoplasm. This union necessitates discharge of eggs and spermatozoa in close proximity, which may be accomplished in water in aquatic animals, or in special cavities of the female, more commonly in land animals. Thus, there are two types of fertilization; **external** and **internal fertilization**.

External fertilization: Particularly in aquatic animals, such as such as echinoderms, many fish and amphibians (frogs), both ova and sperms are laid directly into water where the fertilization takes place, i.e. outside the body (Figure 4). Such type of fertilization is known as external fertilization. It has following advantages over the external fertilization;

- New offspring are exactly like the parent.

- Production of large numbers of gametes is unnecessary.
- Copulation and fusion of gametes is passive, i.e. very little energy required to mate.
- Large numbers of individuals are produced.
- Chances of survival of the offspring are more.

Figure 4. External fertilization in frogs

However, it has some disadvantages too. The disadvantages of external fertilization can be outlined as follows;

- Many gametes do not survive.
- Many eggs are not fertilized.
- Offspring are often not protected by parents. So many of them die.

Internal fertilization: In some other aquatic animals, such as cephalopods and in most terrestrial animals, the male deposits sperms, during copulation, either into the oviduct of the female (as in vertebrates) or into special receptacles called **spermathecae** (e.g., insects, spiders), so that fertilization takes place inside the body of the organism. Such type of fertilization is known as internal fertilization. Embryo is also nourished inside mother. It has following advantages over the internal fertilization;

- Embryo is protected from predators.
- Offspring more likely to survive as many species protect their offspring while they mature.

However, it has also some disadvantages, which are as follows;

- Much more energy required to find mate.
- Fewer zygotes are produced, resulting in less number of offspring.
- More energy is required to raise and care for offspring while they mature.

Internal fertilization is complex process which involves mating of male and female, discharge of spermatozoa in a cavity of a female as mentioned above. The changes occurring in the female reproductive cavity before the fertilization are as follows (human):

- **Arrival of sperms:** Male discharges sperm loaded semen the female cavity (female's vagina close to the cervix during coitus in human). This process is known as insemination (a single ejaculation may contain more than 300 million sperms; highest number of sperms are found in the semen of pigs, i.e. 6000 million). Human semen contains about 60% of secretion of seminal vesicles which contains fructose, amino acids, citrate, sulfate molecules, potassium, phosphorus and a hormone prostaglandin; 30% is the secretion of prostate gland which contains citrate, acid phosphatase, calcium, sodium, zinc,

potassium, protein-splitting enzymes, and fibrolysin (it is an enzyme that degrades blood and tissue fibers); a small percent of mucus secreted by bulbo-urethral (Cowper's gland) and urethral glands; and sperms. It is 7 to 7.5 pH value and can't survive in acidic pH. Sulfate molecules prevent the sperms from swelling.

- **Movement of sperms:** From the site of deposit, sperms travel up the uterus. Out ot millions of sperms, only a few thousand of sperms find their way into the openings of the fallopian tubes. Primarily, sperms are assisted by the contraction of the uterus and fallopian tubes but later they move by their own motility, i.e. by swimming in the fluid medium of fallopian tube at the rate of 1.5 to 3mm per minute. Here many of sperms are engulfed by phagocytic leukocytes of the vaginal epithelium.
- **Arrival of secondary oocyte or ovum:** The secondary oocyte is released from the mature Graafian follicle of an ovary by a process known as ovulation. The oocyte is caught by the fimbriae of Fallopian funnel and sent into the Fallopian tube by the movement of fibrie and cilia. The secondary oocyte can be fertilized only within 24 hrs after its release from the ovary. Here only one sperm succeeds in fertilizing the secondary oocyte. Just after the entry of sperm nucleus, the second meiotic division is comleted.
- **Capacitation of sperms:** The secretions of the female genital tract remove coating substance deposited on the surface of the sperms particularly those on the acrosome. Thus, the receptor sites on the acrosome are exposed and sperm becomes active to penetrate the egg. This phenomenon of sperm activation in mammals is known as capacitation. The secretion of seminal vesicles, prostate gland and bulbo-urethral glands, which neutralized the acidity of female reproductive tract, is mainly responsible for the activation of sperms which takes about 5 to 6 hours.

Species recognition: It is very specific process. The sperms of one species can fertilize the ovum of the same species. It never fertilizes the ovum of other species. This species specificity utmost biological significance and is achieved through specific biomolecules termed as **fertilisin,** which is a glycoprotein 30kD composed of different types of amino acids and monosaccharides according to the species. Both sperm and ovum contains fertilisin on their surfaces. The surface layer of sperm also contains another proteinaceous substance known as **antifertilisin**, which is a protein of 10kD and is composed mainly acidic amino acids. Egg fertilizing of a species can only interacts with antifertilisin present on the sperm of same species. Fertilisin of egg-water also attracts the sperms of the same species and many sperms adhere together. This type of mutual adhesion of the sperms is known as the **agglutination** and is most common in sea urchins (Figure 5).

Process of fertilization: After mating, both mature spermatozoa and oocyte come in contact, interact and fuse their plasma membrane. In this process, many biochemical events take place, which includes two successive steps; the **activation of the egg** and the **amphimixis** which can be outlined as follows:

1. **The activation of the egg:** The activation of egg is a complex process and requires certain biochemical changes. Thus the activation process can be summarized in following three stages:

- **Movement of the sperm towards the egg:** The sperms which occur in the external (external fertilization) and internal fluid (internal fertilization) media around the egg, swim towards the egg at random. They collide with the egg cell by chance. This collision of the sperms with the egg cell occurs regularly. This is fruitful only due to the large number of the sperms and enormously large size of the ovum.

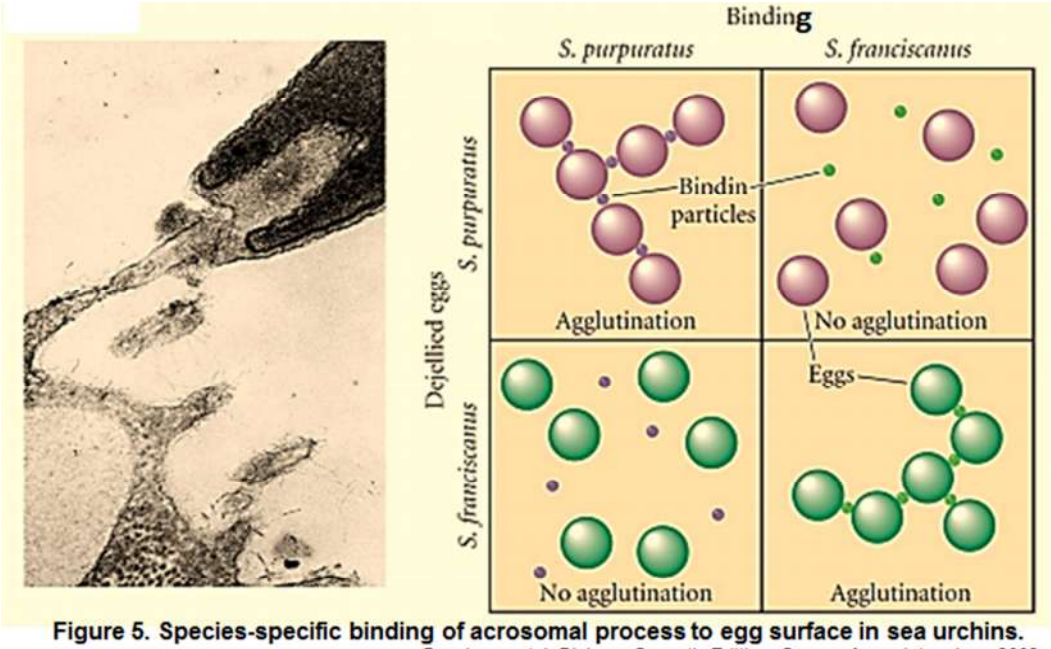

Figure 5. Species-specific binding of acrosomal process to egg surface in sea urchins.
Developmental Biology, Seventh Edition, Snauer Associates, Inc., 2003

The fertilisins and antifertilisins become active after the chance collision of the sperms with the ova. The egg fertilisin usually occurs in the jelly surrounding the egg. It gradually dissolves in the surrounding water of the egg and forms the so called egg water, whereas egg antifertilizin reside on the zona pellucida layer of the egg and binds to fertilisins present on the sperm surface (Figure 6)

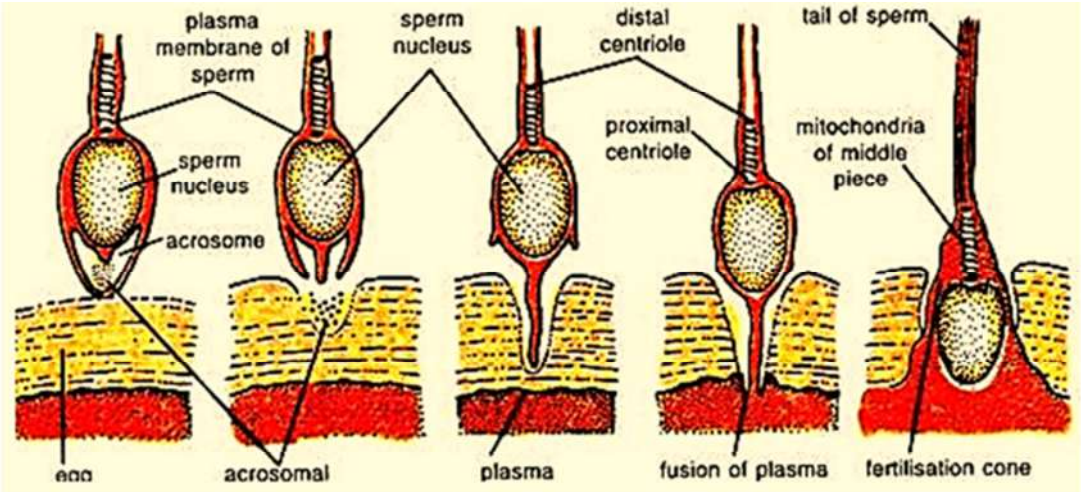

Figure 6. Collision and interaction of sperm with the egg cell.

- **Activation of the sperms:** When a sperm having specific antifertilisin comes in contact with egg water having dissolved fertilisin which results in the significant changes in the acrosome of the sperm. The peripheral portion of the acrosome collapses and release hydrolytic enzymes or lysins, such as hyaluronidase and acrosin. The lysins extruded from the sperm head and dissolve in the egg water and starts digesting zona pellucida layer of egg cell. The central portion of the acrosome elongates and forms a 1 to 75μ long, thin tube acrosomal filament (a rigid filament protruded from the sperm head). The possession of acrosomal filament of the sperm is said to be activated that is ready to be penetrated into the egg jelly and vitelline membrane by the help of dissolving action of the lysisns. Soon sperm rotates to 180° and its middle part comes in contact in front of egg.
- **Activation of the egg and insemination:** As soon as the tip of the acrosomal filament touches the egg membrane (plasma membrane), various important morphological and physiological changes start in the egg. The ooplasm protrudes out at the point of contact into a cone-like process known as the **fertilisation cone** (Figure 6). The fertilisation cone may be conical, cylindrical or irregular. When the fertilisation cone is irregular in shape, it contains many pseudopodia-like processes of the ooplasm. The fertilisation cone is composed of the plasma membrane and hyaline cytoplasm. The fertilization cone engulfs the sperm and the sperm which is surrounded by the hyaline cytoplasm moves inwards. The penetration of the sperm in the egg is known as **insemination**. Immediately after the insemination, a thin membrane known as the fertilization membrane is formed around the plasma membrane of the egg. The fertilization membrane prevents the entrance of further sperms in the egg.

2. **Amphimixis:** During the insemination, the entire sperm may enter in the egg as in the mammals or the tail of the sperm remains outside the eggs as in the echinoderms or in certain cases, the tail and middle piece of the sperm remain outside the egg and only the head and centrosome enter in the egg. This event is species specific. After the entry of nucleus of sperm called as male pro-nucleus swells up by absorbing water from ooplasm.

During the insemination, the entire sperm may enter in the egg as in the mammals or the tail of the sperm remain outside the egg as in the echinoderms. In certain cases as in Nereis, the tail and middle piece of the sperm remain outside the egg and only the head and centrosome enter in the egg and becomes vesicular. The compactly arranged chromatin material of the male pro-nucleus becomes finely granular. The centriole of the sperm is surrounded by the centrosome and microtubules which form aster rays. The male pro-nucleus and the centriole move towards the egg pro-nucleus. The path is prepared by the enzymatic action of the sperm acrosome and is known as penetration path.

In the case of sea urchins and vertebrates, the two pronuclei (male and female) come close to each other and the close contact takes place between the two. The nuclear envelope is broken at the point of contact and the nuclear contents of both pronuclei are intermingled. The endoplasmic reticulum forms a new common nuclear envelope around the both pronuclei and, thus, forms a zygote nucleus. In case of Ascaris, annelids and molluscs, this type of fusion of two pronuclei does not occur. In these animals the centrioles form the achromatic spindle from the

microtubules of the ooplasm and both male and female pronuclei come close to each other, their nuclear envelopes are dissolved.

Thereafter, the paternal and maternal homologous chromosomes get arranged on the equator of the achromatic figure and the first cleavage (mitotic) division of the egg occurs.

Post-fertilization changes in the eggs

- After fertilization, following changes occur in the egg:
- The egg is no more haploid. The egg is now converted to diploid cell known as zygote.
- The zygote becomes ready for the cleavage and for the formation of the embryo.
- The oxygen consumption of the zygote increases enormously.
- The metabolic rate of the zygote increases many fold. Permeability of the plasma membrane increases and subsequently amino acids also increases that increases the volume of the egg, decreases the exchange of phosphate and sodium between the zygote and surrounding medium, diffusion of the calcium ions from the egg starts and the hydrolyzing activities of the proteolytic enzymes increase.
- The protein synthesis is started.

Kinds of fertilization

There are following types of fertilization in the organisms:

1. **Monospermic fertilization:** In this type of fertilization, only one sperm enters in the egg. It occurs in most animals. It is common in the coelenterates, annelids, echinoderms, bony fishes, frogs and mammals.
2. **Polyspermic fertilization:** In this case, many sperms enter in the egg. This condition is referred to as polyspermia. It may be a pathological condition and therefore it is further classified into two –

    I. **Pathological polyspermy:** This type of polyspermic condition occurs in response to certain abnormal condition. This type of egg does not develop further and dies soon.

    II. **Physiological polyspermy:** In this type of polyspermic fertilization, many sperms enter the egg but only one unites with the egg pro-nucleus and the rest are degenerated soon. Such eggs are viable and develop further. Such case is generally found in large yolky eggs such as in molluscs, selachians, urodels, reptiles and birds.

3. **Polyandry:** When two male pronuceli unite with a female pro-nucleus, the condition is known as polyandry. This condition may occur in man and rat. This condition makes a zygote to be triploid which does not give rise to viable progeny. Soon after the birth, the foetus may die.
4. **Polygamy:** When two female (egg) pronuclei unite with single male mro-nucleus, the condition is known as polygamy. It is commonly found in sea urchins, polychaete, worms, urodels and rabbits.

5. **Gynogenesis:** When sperm only activates the egg but its pro-nucleus does not unite with the egg pro-nucleus, the phenomenon is known as gyanogenesis. This condition is largely seen in planarians and nematodes.

Significance of fertilization

— The fertilisation ensures the usual specific diploidy of the organism by the fusion of the male and female pronuclei.
— The fertilisation establishes definite polarity in the eggs.
— The fertilisation provides new genetic constitution to the zygote.
— The fertilisation activates the egg for the cleavage.
— The fertilisation increases the metabolic activities and rate of the protein synthesis of the cell.
— The fertilisation initiates the egg to start cleavage and embryogenesis.
— Fertilisation combines characters of two parents, thus, introducing variations and making the resulting individuals better equipped for the struggle for existence. This happens only in cross fertilisation.

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