Introduction - Fertilization

- Fertilization involves the fusion of two haploid cells namely sperm and ova to produce a new diploid organism.
- **Types of Fertilization:** There are two different types of fertilization:
- **1. External Fertilization:** The fusion happens outside the body. It occurs in the environment. Various physical conditions like temperature, water, soil, humidity, etc., affect the fertilization process.
- **2. Internal Fertilization:** The fusion happens inside the female body. This fertilization is free from changes in physical conditions like temperature, water, soil, humidity, etc. of the environment.

FACTORS INVOLVED IN FERTILIZATION:

- **Chemotaxis:** Some animal eggs attract the sperms by the release of certain chemicals. These chemicals are found in the chorion lining the micropyle. Once the chorion is removed the activities of sperm are ceased. This is found in coelenterates, fishes, insects, etc.
- **Life Span of Gametes:** In External Fertilization life span is short whereas in internal Fertilization is long which can vary from 17 hrs in Rats to 4 years in Turtles inside the female genital tract.
- **Production of an enormous number of sperms:** A large number of sperms are produced to fertilize the lesser number of the egg.
- **Mechanical Juxtaposition of Gametes:** This refers to the mechanical position by which sperm can reach the ovum. It is different from animal to animal like *Sepia* uses an arm to transfer their sperm, copulation in mammals, etc.
- **Synchrony in the Production and Release of Gametes:** The synchronization of male and female maturity and their release to facilitate fertilization. The fertilazation process involves two different phases namely Pre-Fertilization and Post Fertilization.

Step 1: Pre –Fertilization: This refers to the process which occurs before the fusion of gametes.

Step 2: Post- Fertilization: This refers to the process after the fusion of gametes Fusion of male and female gametes produces a new organism in the fallopian tube and its attachment to the uterus of the female body.

PRE- FERTILIZATION

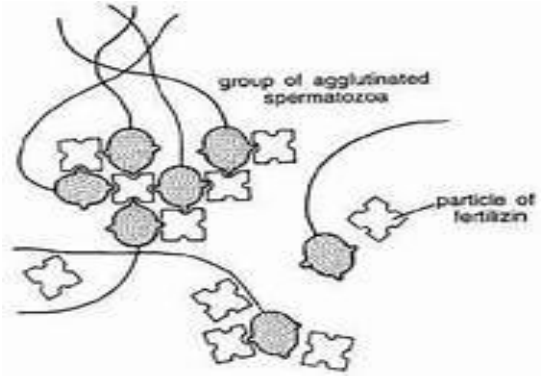
Pre Fertilization comprises the five stages:

STEP 1: Bringing of sperm and ova to the same place:

- Most Invertebrates and vertebrates maintain a close distance between spermatozoa and ovum by the act of some behavior.
- Spermatozoa and ovum need to be in a liquid medium at the same time for fertilization. There are two types of fertilization based on nature and place.
- In External Fertilization, eggs are released on a liquid medium followed by the release of sperm near them.
- In Internal Fertilization they are brought together by an act of copulation.
- In some animals especially where the fertilization is external Sperm are attracted to eggs by the release of chemicals called Chemotaxis.
- These Chemotaxes are released by the egg. These chemicals are present in the chorion lining the micropyle.

STEP 2: Capacitation and contact:

- Capacitation refers to the act where the sperm fertilizes the egg of the same species. There exists a set of chemicals i.e fertilizins and antifertilizins which ensure that fertilization will occur between the gametes of the same species. These chemicals also ensure that only one sperm can fertilize the egg.



Mechanism of Fertilizin and Anti fertilizin Reaction

Fertilizin – antifertilizin reaction:

- **F.R. Lillie** postulated the Fertilizin and Anti Fertilizin Theory.This reaction between Fertilizin and Anti Fertilizin ensures the sperm and ova of a certain species can only mate.
- Fertilizin is the chemical found on the surface of the egg. These Fertilizin molecules have many receptors or binding sites to bind with the sperm.
- These receptors are species-specific to fuse with the sperms of the same species. These glycoproteins molecules are embedded with the jelly coat or plasma membrane of the ovum.
- Anti-Fertilizin is the chemical found on the surface of sperm. They are acid proteins with a smaller molecular weight than fertilizin. This anti-fertilizin is also species-specific.
- The reaction between Fertilizin and Anti Fertilizin is quite analogous to the lock and key mechanism of enzymes.

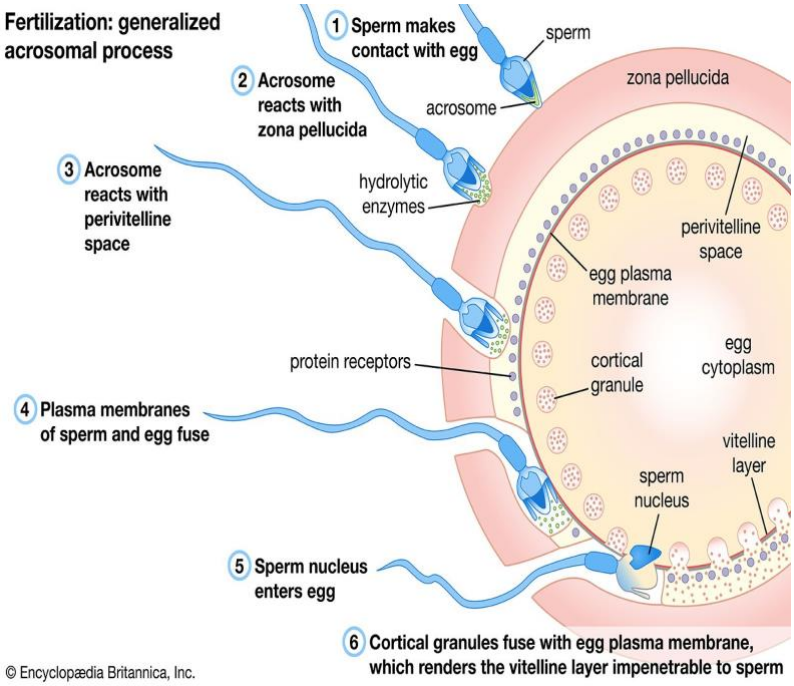
Mechanisms of Fertilizin and Anti fertilizin Reaction:

- Sperms identify the eggs and react by identifying with Fertilizin molecules.
- The first attachment between egg and sperm is due to the link between fertilizin and antifertilizin particles.
- Eggs are released in a liquid medium. Due to the liquid medium, few molecules of fertilizin combine with the medium. These start to attract all the sperms currently in the same medium.
- This aggregation of sperms leads to agglutination. Only a few sperms reach the surface of the egg. This mechanism helps to reduce polyspermy.
- This action ensures that fertilization will be done within species.
- Fertilizin activates the sperm to initiate the acrosome reaction to dissolve the egg membrane.

STEP 3: Acrosome reaction and penetration:

- Eggs (Ovum) are covered with one or more layers of membrane or gelatinous or follicle cell to prevent the egg from getting fertilized except in Porifera and Coelenterates.
- Spermatozoa need to break these layers to make the process of fertilization. Spermatozoa get attached to these layers and become motionless.
- The acrosome found in the head of the sperm performs physiochemical activity to break these layers.

- Acrosome releases a certain enzymatic protein called sperm lysins. In mammals corona radiata acts as a barrier that prevents spermatozoa to reach the ovum.
- To facilitate the sperm acrosome has an enzyme called hyaluronidase which aids in dissolving the adhesive layer and cell disbursement.
- The acrosome undergoes morphological change and transforms into a filament to help the release of sperm inside the egg.



Stages of the sperm-egg association during fertilization

Acrosome reaction and penetration in *Saccoglossus*:

- **Colwin and Colwin,** 1967 described the acrosome reaction and penetration in ***Saccoglossus***.
- In ***Saccoglosus*** spermatozoon have a spherical nucleus, a tail, and an acrosomal vesicle at the front.
- These acrosomal vesicles are surrounded by acrosomal granules except at the apex. Apex has a space filled with a material called peri acrosomal material.

The following events occur when sperm comes in contact with an egg. They are

- **Bursting of acrosome:** Acrosomal apex bursts and expose acrosomal vesicles.
- **Lytic enzymes are released:** Acrosomal vesicles touch the egg layer and release Lytic
- enzymes to make passage through the layer.
- **Acrosomal tubules are formed:** Acrosomal membrane starts to move towards the nucleus and form a long slender tubule.

- **Fusion of acrosomal tubule with egg membrane:** Acrosomal tubule enters the egg after Lytic enzyme has made a passage through the membrane.
- **Sperm Contents are passed:** Acrosomal tubules dissolve and the other material of the sperm gets mixed with the egg cytoplasm. The nucleus of the spermatozoon moves towards the fertilization cone. This process remains the same for other animals. It is the number and size of acrosomal tubules which vary from species to species.

STEP 4: Ovum activation:

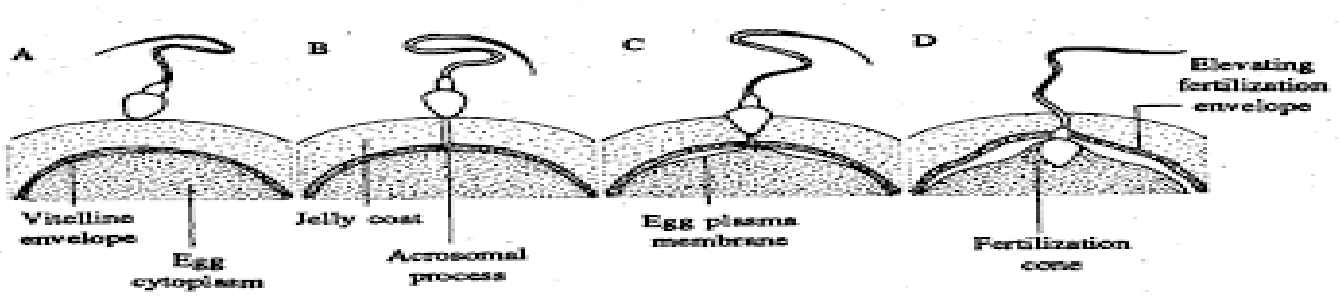
- This process starts when the sperm touches the surface of the egg plasma membrane facilitated by acrosome filament.
- The fusion of the two eggs takes place and a single mosaic membrane is formed. Thus plasma membrane of both cell become a single cell called zygotes.
- A lot of changes occur in the cytoplasm of the egg. These changes have been summed up in the following steps:

1. Fertilization Cone Formation.

2. Formation of membrane and cortical reaction.

1. Fertilization cone formation:

- The acrosomal filament of spermatozoa touches the egg membrane, and the cytoplasm of the egg moves forward at the point of contact and forms a structure that appears like a simple conical protrusion.
- The cytoplasm started to engulf the spermatozoa. Spermatozoon doesn't enter the egg cytoplasm intact instead they are spread over.
- The Sperm nucleus and other sperm structures move towards the fertilization cone. The plasma membrane of spermatozoa mixes with the egg plasma membrane to form a single entity.



Fertilization cone formation

- The acrosomal granule stays outside the egg membrane; only peri acrosomal material enters the egg cytoplasm.

- Some variation exists in how the spermatozoon is taken into the interior of the egg. In mammals, the entire spermatozoon penetrates the egg cytoplasm.
- In ***Nereis*** only the head and proximal centriole enter the egg cytoplasm. However, as a golden rule in the majority of animals, the sperm nucleus and mid-piece enter the egg.

2. Formation of membrane and cortical reaction:

- A set of physical-chemical reactions occur inside the egg before the spermatozoa start to penetrate them. The set of physical-chemical reactions is known as a cortical reaction.
- This cortical reaction role is concerned with the formation of membrane outside the egg plasma with the sole objective to prevent the arrival of late spermatozoa inside the egg.
- The process of membrane formation varies from animal to animal. A glimpse of cortical reactions and membrane formation in some animals has been discussed.

Sea Urchins:

- Upon touching of acrosomal tubule with egg surface, color changes from yellow to white (under dark field microscopy), which travels rapidly around egg cortex.
- It is followed by the formation of a fertilization cone and membrane around the egg plasma membrane.
- Sea Urchin unfertilized egg has egg cortex bound by two membranes namely vitelline and inner thick plasma membrane.
- A layer is formed below the plasma membrane. The fertilization membrane is formed in the following way:
- Outer vitelline membrane is separated and undergoes an expansion to become the outer layer.
- During expansion cortical granules explode and release three components namely Dark, denser, lamellar, and folded parts, Globules, and the liquid component.
- All the three component form different structures namely

1. Dark, denser, lamellar, and folded part: They fuse with the inner side of the vitelline membrane.

2. Globules: They form the surface of the hyaline layer. This hyaline help to keep the

- blastomeres together.

3. Liquid component: They fill the perivitelline space between the new egg surfaces. It contains mucopolysaccharides and water.

- All three components together form a fertilization membrane (Slow block to polyspermy)

Vertebrates:

- Upon touching of acrosomal tubule with the egg surface, Lytic digestive enzymes are released by the acrosomal tubule to penetrate the egg membrane.
- The cortical granules are broken into egg cytoplasm and their content becomes liquefied and comes on the surface and fills the perivitelline space between the chorion and plasma membrane.
- In fishes chorion becomes hardened. In mammals, cortical granules fill the space between egg plasmalemma and the zona pellucida.
- An animal that lacks cortical granules such as urodele amphibians, some lower order mammals.
- In those animals neither a membrane is formed nor does neither any cortical reaction take place.

Theory of Activation:

- Several Theories have been proposed to illustrate how sperm stimulates the eggs. They are
- **Theory of Boveri:** Boveri proposed that the mature egg has no active division center. This division center is transferred into the egg by the sperm to initiate the process of cell division.
- **Theory of Loeb:** Loeb, 1913 proposes two principles namely the lytic principle and cytolysis principle. The lytic principle states the factors which increase the oxidation process and the cytolysis principle deals with controlling the increased oxidation rate.
- **Theory of Bataillon:** Bataillon proposed that the secretion of a substance in the perivitelline membrane and elevation of the fertilization membrane activate the egg to start fertilization.
- **Viscosity Theory:** Heilbrunn postulated that calcium is released during fertilization which increases the viscosity of egg cytoplasm. This increased calcium concentration starts the development process.
- **Deinhibition Theory:** Runnstrom and Brachet observe that metabolic inhibitors accumulate in the oocyte during the maturation process. These inhibitors are removed and eggs are deinhibited.

Step 5: Pronuclei migration and amphimixis:

- The sperm nucleus remains compact with mitochondria and centrioles behind it after penetrating the membrane of the egg. The sperm nucleus has to perform two functions to be able to perform amphimixis.

1: Becomes pronuclei.

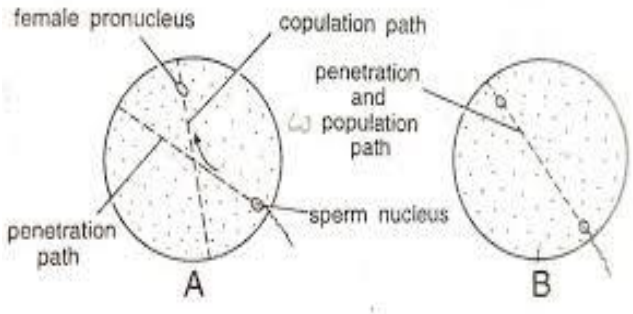
2: Migrate from the site of amphimixis.

1: Becomes Pronuclei:

- After the penetration of the egg by spermatozoon, the nucleus starts to move inwards from the site of cone formation.
- The nucleus rotates by 180o to enable mitochondria and centrioles to assume the first position.
- The sperm nucleus starts to swell. The chromatin material inside the cell starts to become granular. It starts to look more like an interphase nucleus called a male pronucleus.

2: Migrate from the site of amphimixis:

- Sperm centriole is surrounded by sperm aster in the egg cytoplasm. Sperm aster starts to lead the nucleus to the site of amphimixis (fusion of male and female gametes).



Copulation Path

- The egg nucleus also undergoes some changes. The Haploids nucleus of the egg fuses into one another and forms a female pronucleus. They also swell and become vesicular.
- The female pronucleus also starts to move towards the site of amphimixis.
- The site of fusion lies near the center of the microlecithal and telolecithal egg or at the center of the active cytoplasm.
- The path that which male pronucleus follows depends upon the effect of the
- chemical released by the female pronucleus.
- The normal path followed by the male pronucleus is known as the penetration path and a new path subjected to some changes is known as the copulation path.

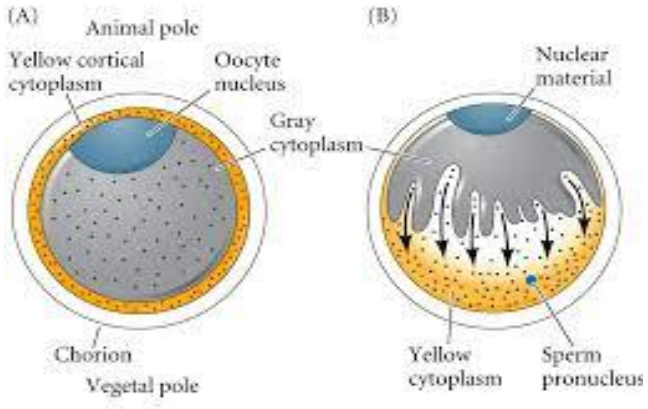
POST FERTILIZATION

- Spermatozoa penetration of the egg results in a significant change in the position of cytoplasmic constituents.
- Several new areas emerge within the egg. Cortical granules extrusion results in replacing the outer egg cell surface with the inner surface with everted on the exterior.
- The most significant change in the cytoplasm has been observed in ascidian and frog. A bilateral symmetry has been established in the cytoplasm of both animals in the fertilized egg.
- A ***Zygote*** is formed after the rearrangement of the cytoplasm. Displacement has been quite remarkable in the ascidian, frog, and ***Styela partita***. This need arise to rearrange the nucleus and genetic material equally.

Displacement of cytoplasmic material in ascidian *Styela partita*:

- The mature egg of ***Styela partita*** is covered with a layer of cortical cytoplasm which contains yellow granules. As the spermatozoon enters the egg, the cytoplasm starts a violent commotion.
- The cytoplasm starts to move towards the vegetal pole and arrange itself into the shape of a cap.
- As sperm move towards the egg nucleus, the cytoplasm moves upward towards the direction from where spermatozoa entered. This start to enter four different regions of the cytoplasm. They are

  1. Yellow cytoplasm on one side.
  2. Light color cytoplasm on the other side.
  3. Slaty grey color cytoplasm containing yolk granules and mitochondria.
  4. Clear and transparent Cytoplasm



Displacement of cytoplasmic material in ascidian Styela

- This displacement separates the cytoplasm and arranges them to start the process of cleavage.
- Fertilization ensures that the cycle of cleavage can start, which will become the first step of embryo development. This is followed by morulation, Blastulation, Gastrulation and finally, a new multicellular organism is brought into the world.

BIOCHEMISTRY OF FERTILIZATION

Metabolic activation:

A series of cytoplasmic reactions take place after the sperm penetrates the unfertilized egg. The steps involved are:

a. Changes in plasma membrane

b. Ionic changes

c. Changes in the rate of respiration

d. Co-enzyme changes

e. Rate of protein synthesis

f. Mitosis initiation

g. Breakdown of polysaccharides

h. Increase in hexose phosphate

i. Dehydrogenase

a. Changes in the plasma membrane:

- The sperm penetration has increased the movement of water and other chemicals like ethylene, glycol, phosphate, K+ , etc. This led to the increase of electrical potential in the plasma membrane.
- It became more positive, but slowly turns negative due to unequal distribution of chloride ions.
- Adenyl cyclase, an enzyme of plasma membrane gets activated at the time of fertilization and initiates the formation of 3’ – 5’ cyclicAMP molecule.
- This molecule is responsible for activating metabolic reactions in a fertilized egg.

b. Ionic changes:

- A substantial level of changes in the intracellular composition of the fertilized egg takes place at the ionic level. This is especially in sodium, potassium, and calcium.
- This change in ion concentration had a great influence on the metabolism activation of the fertilized egg.

c. Changes in the rate of respiration:

- The rate of respiration varies from animal to animal. In some animals where fertilization is completed **(Sea Urchin**), it increases whereas in others it either decreases (**Molluscs**) or remains stable (***Bufo***).
- The increased rate is associated with the increased oxygen demand for oxidation of glycogen and the release of numerous ATP molecules.

d. Co-enzyme changes:

- Spermatozoa contain an acrosome that has a lot of oxidative enzymes.
- These enzymes come inside the egg after fertilization and ensure an increase in oxidation. This increase in oxidation is to provide energy for the development of egg and supplement other changes necessary for it. The following process takes place:

NAD + ATP NADKinase NADP + ADP

e. Rate of protein synthesis:

- The cytoplasm of an unfertilized egg contains a lot of protein synthesis material like DNA molecules, tRNA, mRNA, ribosomes, and proteolytic enzymes.
- During fertilization, proteolytic activity increase to remove the inhibitor protein and mRNA is unmasked to start the process of active protein synthesis.

f. Mitosis initiation:

- The process of active protein synthesis increases the rate of DNA synthesis. The sperm initiate the first mitotic division (Cleavage) by contributing centriole to the egg.
- Though centriole is there in the unfertilized egg, they are incapable of performing mitotic division.
- This sperm centriole becomes the basics of the new organism gender as half of DNA material is contributed by sperm and another half by egg.

g. Breakdown of polysaccharides:

- An increase in lactic acid concentration takes place on account of the rapid breakdown of polysaccharides.

h. Increase in hexose phosphate:

- This chemical increases considerably after fertilization.

i. Dehydrogenase: The activity of the enzyme performing dehydrogenase increase significantly after fertilization.

SIGNIFICANCE OF FERTILIZATION:

- Maintain diploidy in the race.
- Bring genetic variation.
- Egg is activated to start the process of Cleavage.

Previous year questions

1. In mammalian fertilization ,the acrosome reaction is triggered by

a) Binding of sperm to the zona pellucida

b) Fusion of sperm and egg plasma membranes

c) Cortical granule exocytosis

d) Pronuclear fusion

Correct Answer : (A)

Explanation:

The acrosome reaction releases hydrolytic enzymes (e.g., hyluranidase, acrosin) to penetrate the zona pellucida,a glycoprotein layer around the egg.

2)Which one of the following statements about the cortical reaction in sea urchins is correct?

a)The entry of Ca^{2+} ions into the egg initiates development.

b)The exocytosed cortical granules during egg maturation contain the components of the zona pellucida.

c) The release of the cortical granules after sperm entry converts the vitelline membrane into the fertilization membrane which blocks polyspermy.

d) The depolarization of the plasma membrane after sperm entry helps to block polyspermy.

Correct Answer: d

Explanation:

The cortical reaction (slow block) involves cortical granule exocytosis, leading to the formation of the fertilization envelope from the vitelline membrane to prevent additional sperm entry.

3) In the body of a female,which type of fertilization occurs?

a) External

b) Internal

c) Asexual

d) Both (a) and (b)

Correct Answer : (b)

Explanation:

In mammals and birds, fertilization is internal within the reproductive tract.

4) External fertilization occurs in

a) Humans

b) Amphibians

c) Mammals

d) Dogs

Correct Answer : (b)

Explanation:

Aquatic environments like those of frogs allow external fertilization

5) Amphibian oocytes remain for years in the diplotene stage of meiotic prophase. Resumption of meiosis is initiated by

a) Gonodotropic hormone

b) Growth hormone

c) Oestrogen

d) Progesterone

Correct Answer : (d)

Explanation:

Progestrone from the follicle triggers meiotic resumption in amphibians.

6) In animal cells, typically which organelle is only provided by the sperm to the oocyte following fertilization?

a) Nucleolus

b) Peroxisomes

c) Mitochondria

d) Centrioles

Correct Answer: (d)

Explanation:

Oocytes lack functional centrioles; sperm provides the paternal centriole for mitotic spindles.

7) Consider the following features important for the process of fertilization:

A. Sperm undergo acrosome reaction to penetrate the egg's zona pellucida.
B. B. The fusion of sperm and egg membranes triggers the completion of the egg's second meiotic division.
C. Cortical granules in the egg modify the zona pellucida post-fertilization to prevent polyspermy.

D. Capacitation is a biochemical change that sperm undergo in the female reproductive tract to gain the ability to fertilize an egg. E. The cortical reaction is initiated by the influx of calcium ions post fusion of sperm and egg membranes.

Which option below has all correct features regarding fertilization?

(a) A and B
(b) (b) A, B, and C
(c) (c) B, D, and E
(d) (d) All statements are correct

Correct Answer: (d)

Explanation: These describe key mammalian fertilization steps, including blocks to polyspermy.

8) Sperm lysin, the enzymatic substance produced by sperms in mammals, is known as

(a) Cryanogamone
(b) Hyaluronic acid
(c) Androgamone
(d) Hyaluronidase

Correct Answer: (d)

Explanation:

Hyaluronidase helps digest the protective layers around the egg for sperm penetration.

9) Egg development without fertilization is known as

(a) Parthenogenesis

(b) Metagenesis

(c) Gametogenesis

(d) Oogenesis

Correct Answer: (a)

Explanation:

This is a form of asexual reproduction where the egg develops into an embryo without sperm fusion.

10) After a sperm has penetrated an ovum in the process of fertilization, entry of further sperms is prevented by

(a) Condensation of yolk

(b) Development of the vitelline membrane

(c) Formation of the fertilization membrane

(d) Development of the pigment coat

Correct Answer: (c)

Explanation:

This is the fast block to polyspermy in many animals, triggered by cortical granule exocytosis.

11)In the process of fertilization, this is true

(a) The entry of sperm activates the egg for completing meiosis

(b) Only one sperm reaches the egg and enters it

(c) Only the acrosome of the sperm enters the egg

(d) Two haploid nuclei fuse and immediately divide to produce two nuclei, which are again haploid

Correct Answer: (a)

Explanation: Sperm entry triggers the completion of meiosis II in the secondary oocyte.

12) In rabbits, humans, and other placental mammals, fertilization occurs in

(a) Vagina

(b) Ovary

(c) Fallopian tubes

(d) Uterus

Correct Answer: C

Explanation: Fertilization typically happens in the ampullary-isthmic junction of the oviduct.

13) This helps in the penetration of the egg by the sperm

(a) Fertilization membrane

(b) Antifertilizin

(c) Sperm lysin

(d) Fertilizin

Correct Answer: (c)

Explanation: Sperm lysins (e.g., hyaluronidase) dissolve the egg's extracellular matrix.

14) In mammals, which one of the following proteins is crucial for the recognition and binding of the sperm to the egg, facilitating fertilization?

(a) Bindin

(b) Resact

(c) Izumo

(d) Ovastacin

Correct Answer: ©

Explanation: Izumo1 on sperm interacts with Juno on the egg for membrane fusion.

15) Following statements are made about fertilization occurring in sea urchins:

A. Chemoattraction of the sperm to the egg is mediated by sperm activating peptides like bindin.

B. Exocytosis of the sperm acrosomal vesicles and release of enzymes occur.

C. The capacitated sperm undergoes acrosome reaction.

D. The acrosome protein mediating the critical species-specific binding event is resact.

E. The slow block to polyspermy is accomplished by the cortical granule reaction.

Which one of the following options represents the combination of all correct statements?

(a) A and B only
(b) (b) A, B, and D
(c) (c) B and E only
(d) (d) A, B, and E

Correct Answer: (c)

Explanation: Bindin mediates binding (not chemoattraction), react is a sperm attractant, and acrosome reaction occurs without prior capacitation in sea urchins.