Pre University Examination 2017-18
B.Sc. (I) Zoo
Paper –III (Gamete and Dev Bio)

1. (a) What is biogenetic law? **(1*10)**

Ans. According to"Biogenetic Law" A developing mammal would first be a fish, then an amphibian and then a reptile before it becomes a mammal.

(b) Name the cell organelle from which axial filament and acrosome are formed.

Ans. Axial filament Centrosome, Acrosome: Golgibody

(c) What is spermatogenesis?

Ans. Formation of sperm

(d) Define Parthenogenesis.

Ans. Without fertilization development of embryo.

(e) Write name of egg membranes found in the egg of Hen.

Ans. Discoidal

(f) Define cleavage.

Ans. Initial chain of cell division.

(g) What is teratogenesis?

Ans. Formation of teratogen or abnormal embryo.

(h) What is ageing?

Ans. Changes in metabolic activity.

(i) Give two examples of superficial implantation.

Ans. Dog and Cat

(j) Define Morula.

Ans. 16 cell stage of embryo.

Part-II

Section-A

2. Describe mechanism of fertilization with suitable diagram.

Ans. Fertilization is the fusion of a male and female gamete; spermatozoa and ovum respectively. It results in the formation of zygote from which new offspring is formed. Fertilization fundamentally performs two functions:

(1) It activates the egg to start development.
(2) It injects a male haploid nucleus into the egg cytoplasm. The fusion of two haploid nuclei spermatozoa and ovum, restores the diploid state and introduces genetic variation in the new organism. The intermingling of the paternal and maternal hereditary characters in the offspring is known as amphimixis.

In majority of aquatic animals, the sperms and ova are shed into the surrounding water where fertilization takes place. It is called external fertilization. Examples: fishes, amphibians and echinoderms. In amniotes, the male introduces sperms into the female's tract where the fusion takes place. It is called internal fertilization. This mode of fertilization takes place in animals whose development is ovoviviparous (e.g. certain lizards and snakes, scorpion, some-fishes) or viviparous (e.g. some fishes like *Musculus*, marsupials and placental mammals including man). Internal fertilization is the rule in those viviparous animals which lay eggs covered by hard shells (e.g. insects, most reptiles, birds and egg-laying mammals). Animals in which internal fertilization takes place posses specialized sex organs for transmitting and receiving the sperms. In such forms the fertilization may occur either in the lower part of the oviduct (e.g. Urodeles); in upper part of the oviduct (e.g. salamanders, reptiles, aves and most mammals); or in the ovarian follicles (e.g. viviparous fishes like *Gambusia affinis* and certain eutherian mammals such as *Ericulus*).

The process of fertilization in animals is completed through different 5 steps:

1. The meeting of gametes.

2. Barrier Penetration.

3. Sperm and egg fusion: the acrosome reaction.

4. Activation of the ovum.

5. Migration of pronuclei and amphimixis.

1. The Meeting of Gametes

Whether fertilization is external or internal, the first step is the encounter of spermatozoa and the ovum which is brought about by the swimming movements of the spermatozoa. The movements of the spermatozoa are entirely at random and they collide with the egg as a matter of pure chance. However, in all those vertebrates in which fertilization is internal, spermatozoa tend to be transported passively, from the site of deposition to the site of fertilization, by muscular contractions of the female genital tract.

(a) Agglutination: In most animals it has been observed that in the presence of ripe eggs, the spermatozoa adhesion of spermatozoa to each other result in their clumping or agglutination.

(b) Fertilizin-Antifertility reaction: Lillie (1919) was the first to show that the eggs produce a substance called fertilizin which combines with a substance termed antifertilizin present on the surface of spermatozoa. As a mater of fact, fertilizin is released in the surrounding water and, as a consequence of combining reaction with antifertilizin, most of the sperms agglutinate or clump together.

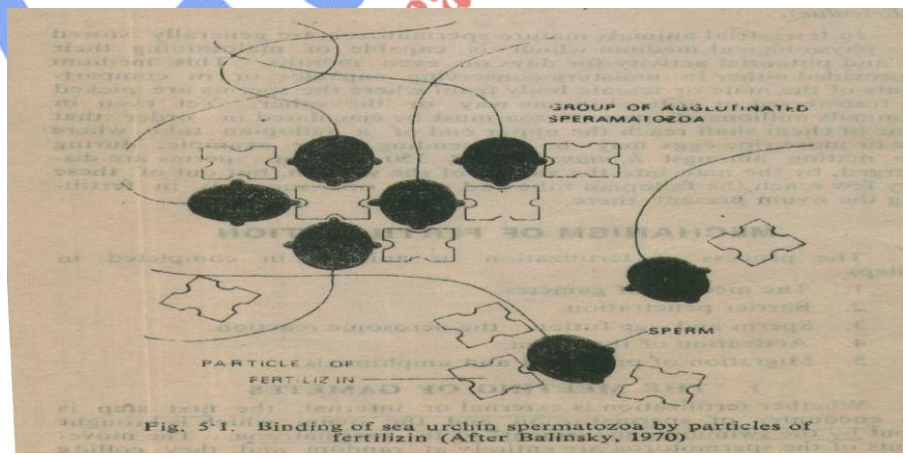


Fig. 5·1. Binding of sea urchin spermatozoa by particles of fertilizin (After Ballinsky, 1970)

Fig. Binding of sea urchin spermatozoa by particles of fertilizing

Chemically, fertilizin is a glycoprotein which consists of a number of amino acids and one or more monosaccharides (glucose, fucose, fructose, or galactose). Depending upon their chemical composition, there are many types fertilizin in different animals.

The molecules of the fertilizins are quite large Molecule. The antifertilizins are acid proteins with a fairly smaller molecule.

The adhesion of the spermatozoa to the surface of the egg is brought about by linking of fertilizing molecules with antifertilizin molecules percent on the surface of the spermatozoon. This phenomen on established an initial bond which would later lead to the penetration of the sperm into the egg. This reaction is highly specific that is fertlizin of one species will not react with the antifertilizin of another species. This is analogous to a lock-and-key-effect, and serves as a barrier against cross breeding. It qualifies the spermatozoa to become fully capable of fertilizing an egg of the same species. Berill (1971) called it capacitation.

2. Barrier Penetration

The surface of the ripe egg is rarely naked (as in coelenterates). It is usually surrounded by egg membranes or follicle cells or both (as in mammals). The spermatozoa has to penetrate through these barriers before it can reach the interior of the egg. The mechanism of penetration is chemical. The egg envelopes are by substances of an enzymatic nature known under the general name of spermlysins released by the acrosome of the sperm.

In the case of eggs with very thick and resistant membranes e.g., fishes and insects, the sperm penetrates through a special canal, the micropyle which is left in the egg membrane.

In mammals, the egg when released from the ovary, is commonly encased in a layer of cells of ovarian origin, termed the corona radiate. These cells are held together by a cementing substance known as hyaluronic acid. The spermatozoa has to pass through this barrier on its way to the egg. In order to do so, its acrosome produces and enzyme hyaluronidase which dissolves the cement and disperses the cells. The enzyme thus enables the spermatozoa to penetrate the corona radiata.

3. Sperm and egg fusion: The acrosome reaction

As the spermatozoa comes in contact with the egg, the acrosome of the sperm undergoes radical changes as the spermatozoa makes contact with the egg envelope, the sperm plasma membranes and the acrosomal membrane rupture at the point of contact.

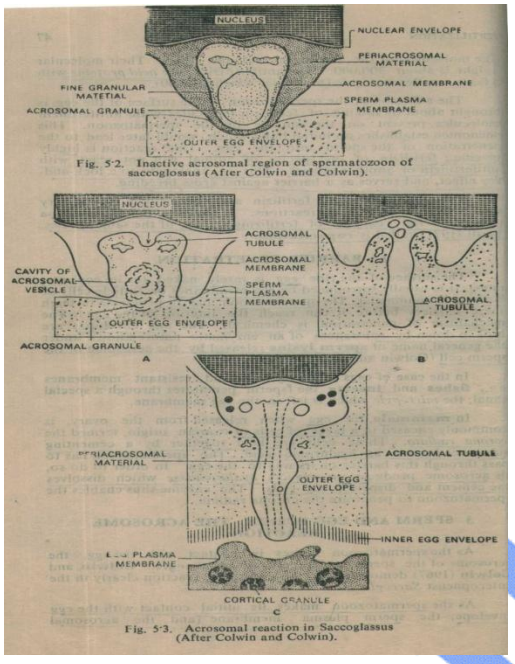


Fig. 5·2. Inactive acrosomal region of spermatozoon of saccoglossus (After Colwin and Colwin).

Fig. 5·3. Acrosomal reaction in Saccoglassus (After Colwin and Colwin).

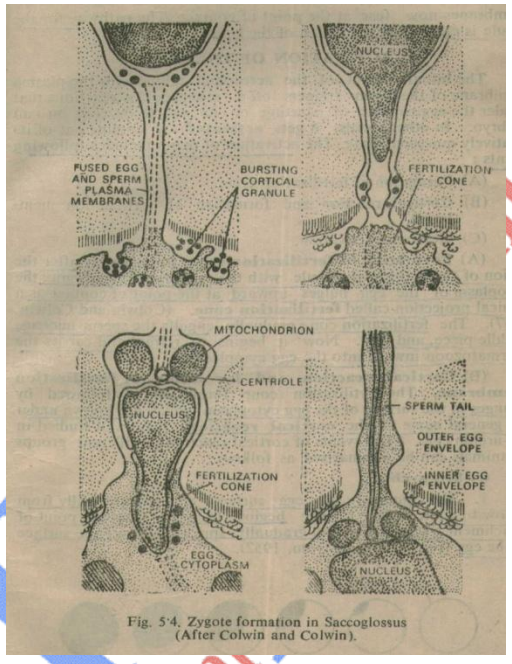


Fig. 5·4. Zygote formation in Saccoglossus (After Colwin and Colwin).

The acrosomal membrane then joins with the plasma membrane the margin of rupture so that the acrosomal granule is exposed to the surface of the egg envelope. Now the peripheral part of the acrosome collapses and its lysins (enzyme) are extruded. The central part of the acrosome elongates and becomes transformed into a long thin tube known as the acrosomal tubule. The acrosomal tubule traverses the egg envelopes (jelly and vitelline membrane) and finally touches the egg plasma membrane. These two membranes now fuse as the point of contact. The pathway for the tubule is cleared by the action of the lysins.

4. Activation of the ovum

The initial contact of the acrosomal tubule with the plasma membrane of the ovum triggers off a sequence of reactions that render the egg capable of starting on its way to develop into an embryo. In other words, it gets activated by coming out of its relatively quiescent state. The activation of ovum includes following events.

(a) Formation of fertilization cone

(b) Cortical reaction and formation of fertilization membrane.

(c) Metabolic activation.

(a) Formation of Fertilization cone: Immediately after the fusion of the acrosomal tubule with the egg plasma membrane, the cytoplasm of the egg bulges upward at the point of contact as a conical projection called fertilization cone. The fertilization cone gradually engulfs the sperm. Now it begins to retract carries the spermatozoa in ward the egg cytoplasm.

(b) Cortical reaction and formation of fertilization membrane: The fertilization cone formation is followed by changes in the surface of the egg cytoplasm which are known under the general name for the cortical reaction. The events of cortical reaction is different in different groups of animals.

1. The colour of the egg surface changes gradually from yellow to white sea-urchin. The cortex of the unfertilized egg is bounded by two membranes. The outer one, called the vitelline membrane whereas the inner one being the plasma membrane. A layer of cortical granules is found beneath the plasma membrane. The outer, vitelline membrane becomes lifted off from the plasma membrane. It undergoes an expansion, an expansion, and gives rise to the outer layer of the fertilization membrane. The space between it and the surface of the egg is called perivitelline space. The cortical granules swell and explode.

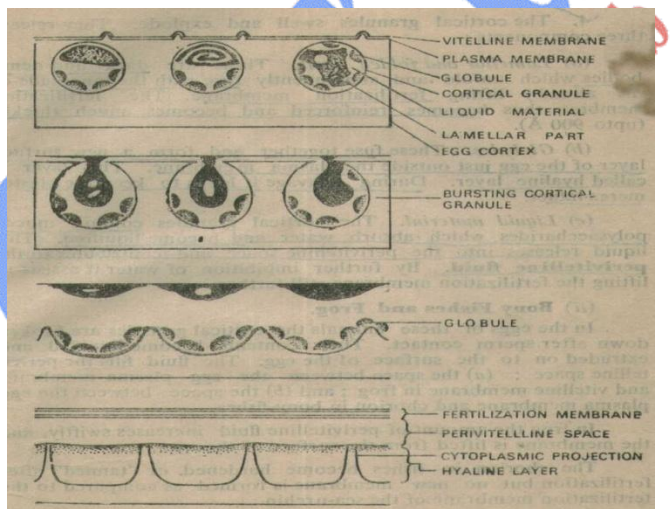


Fig. 5·6. Cortical reaction and the formation of fertilization membrane in the egg of sea-urchin.

Fig. 5·7. Mechanism of the cortical granule break-down and elevation of ferrilization membrane in the sea-urchin egg.

Fig. Mechanism of the cortical granule break –down and elevation of fertilization membrane in the sea-urchin egg.

Eggs of all mammals do not process cortical granules. When cortical granules are present as in human, hamster and the rabbit; theses, release their contents into the space between the egg and zona pellucida (the peri space) which are gradually dissolved. Therefore, no new membrane is formed around the egg at the time of fertilization.

(C) Metabolic Activation: The visible structural changes during fertilization are accompanied by alteration in he physiological properties of the egg substance. These include (i) changes in permeability of egg plasma membrane, (ii) ionic changes, (iii) respiration changes, (iv) change in the rate of protein synthesis, and (v) initiation of mitosis.

5. Migration of pronunclei and Amphimls (Union of Haploid Nucle):

In the case of most vertebrate eggs, oogenesis comes to a standstill after the first meiotic division and is resumed only following sperm penetration. Entrance of spermatozoa serves to act as a stimulus which causes the second maturation division. As the head and middle piece of the sperm advance into the egg, these parts rotate through an angle of 180^0 so that the mitochondria and proximal centriole of the associated middle piece assume the leading position. The nucleus itself starts swalling by absorbing fluid from the surrounding cytoplasm and becomes vesicular. It is now called male pronucleus.

As the male pronucleus and centroile move inward, they may be accompanied by some cortical and subcortical cytoplasm. If the latter is heavily pigmented, as in symphysion eggs, the path of the male pronucleus may be marked by pigment granules trailing along its path. This path is referred to as penetration path.

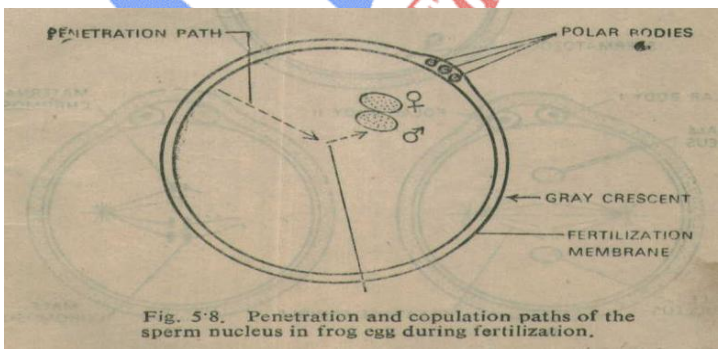


Fig. 5·8. Penetration and copulation paths of the sperm nucleus in frog egg during fertilization.

Fig. Sequence of evens in fertilization (semi diagrammatic) (A) First polar body formed and second in process of forming upon entrance of sperm. (B) Second polar body formed and

pronuclei approaching each other. (C) Maternal and paternal chromosomes arranged on spindle in preparation for first cleavage

The centriole brought in by the permatoza subdivides into two and an achromatic spindle is established in the centre of the active cytoplasm.

With the production of the second polar body the egg nucleus or female pronucleus is ready for union with the male pronucleus provided by the sperm head. It also migrates towards the site of union. Both pronucleri assume the general form of an interphase nucleus.

The male pronucleus which has been advancing along the penetration path, now moves directly toward the female pronucleus. This in many cases involves a slight change in the course of sperm. In such cases, the latter portion of its course is called the copulation path as distinguished from the first portion of entrance path.

Amphimixis: The fusion of male and female pronuclei is called amphimixis. The site of amphimixis lies in the centre of active cytoplasm at the animal pole in macro and telolecithal eggs, while in microlecithal eggs it lies near the centre of the egg.

In a few animal types, the two pronuclei actually fuse together. In mammals and other vertebrates the male and female pronuclei do not fuse as such.

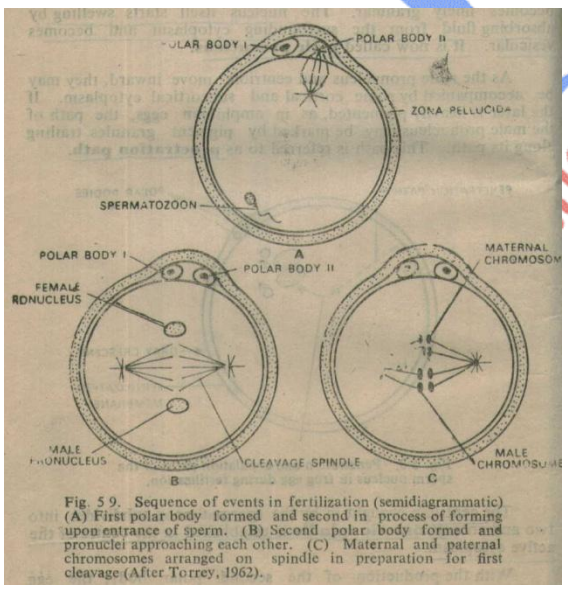


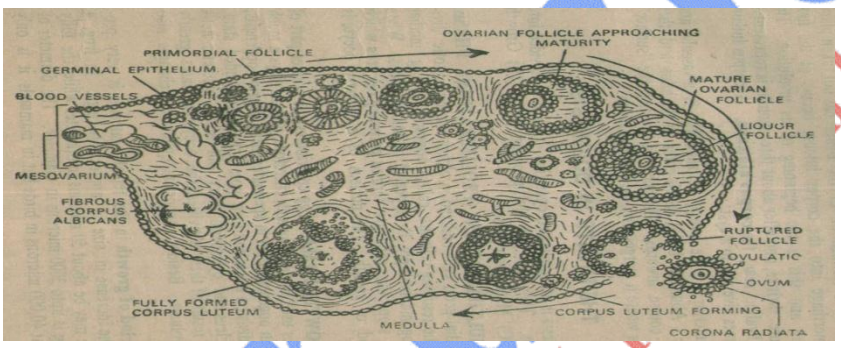
Fig. 5 9. Sequence of events in fertilization (semidiagrammatic) (A) First polar body formed and second in process of forming upon entrance of sperm. (B) Second polar body formed and pronuclei approaching each other. (C) Maternal and paternal chromosomes arranged on spindle in preparation for first cleavage (After Torrey, 1962).

Instead, each pronucleus loses its membrane and its chromation resolves into the paploid set of chromosomes. The two sets of chromosomes then arrange themselves across the division spindle. This arrangement heralds the readiness for the first cleavage division.

3. Write a detail account on growth phase during oogenesis.

Formation of ovum form primordial germinal epithetical cells is known as oogenesis. The process is divided into three phases (A) Multiplication Phase (B) Growth Phase and (C) Maturation Phase

A. Multiplication (Proliferation) Phase:
The primordial germ cell undergoes proliferation by mitotic divisions and the resulting cells are called oogonia or egg-mother cells. The oogonia multiply by repeated mitotic divisions. When the division stops, the cells are termed primary oocytes which enter a period of growth. The nucleus of a primary oocyte is diploid.



B. Growth Phase:
Egg contributes the greater part of the substances used in development, therefore, growth plays a much greater role in oogenesis than in spermatogenesis. During oogenesis, first meiotic division starts and then goes into a suspended state, while the nucleus and cytoplasm carry out major synthetic activities. As a result of these activities, the oocyte increases greatly in size and volume. Besides, important qualitative changes also occur.

The period of growth in the female gametes is very prolonged and the increase in size is appreciably high. In frog, a young oocyte may be about 50 microns in diameter while the fuly developed egg is up to 2000 microns in diameter. The diameter of ovum is about 40,000 microns in birds but in mammals it is only 200 microns.

Period of oocytes may be divided into two stages (i) Previtellogensesis and (ii) Vitellogenesis (Raven,1961).

(i)Pre-vitellogenesis: During this period of growth, the nucleus and cytoplasm of primary oocyte increases tremendously in volume.

(a) Growth of nuclear substances: The nucleus of the oocyte becomes enlarged mainly because of the production of a large amount of nuclear sap. Due to this the nuclei of advanced oocytes appear to be bloated with fluid an are often referred to as germinal vesicles.

The chromosomes increase in length. In oocytes of animals having large eggs(notably in the amphibian oocyte), the chromosomes acquire a very characteristic appearance. Numerous paired threads or loops project transversely from the main chromosomal axes. The shaggy appearance given to the chromosomes by these loops has led to their being called lamp brush chromosomes.

The loops of lamp brush chromosomes represent loci of gene activity i.e., at these sections messenger RNA is synthesized which subsequently controls the synthesis of proteins in the cell. However, the amount of DNA in the chromosomes does not increase in proportion to the enlargement of the nucleus.

The nucleus in the germinal vesicle is concerned with the synthesis of ribosomal RNA. The nucleolus of a growing oocyte increase greatly in size. In many animals, for example in amphibians, instead of one large nucleolus, many smaller nucleoli are formed in the germinal vesicle. It is believed that RNA passes out of the nucleus into the cytoplasm during the growth of the oocyte.

(b) Growth of cytoplasmic substances: The amount of cytoplasm increase quantitatively during the growth of the oocyte. Besides, it also changes in quality by elaboration and regular distribution of various cell inclusions like motochondria, golgibodies, endoplasmic reticulum, cortical granules etc.

Mitochondria: The mitochondria are fewer in young oocytes but increase in numbers quite appreciably during the growth of oocytes. In some animals, e.g., amphibia, birds, they are aggregated in the form of large mitochondrial cluds (Romanoff, 1960; Wartenberg, 1962; Balinsky and Devis, 1963). Mitochondria are carriers of oxidative enzymes, therefore, the overall oxygen consumption increase during the growth of the ooyte.

Golgibodies: younger ooytes, golgi bodies are found around the centrosome. In mature oocytes the sometimes disappear completely (Odor,1960). This indicates that golgi bodies are transformed into some other structures like cortical granules.

Endoplasmic reticulum: The cytoplasm of the young oocytes contains numerous vesicles surrounded by a simple membrane. Ribosomes are often attached to the surface of the vesicles. These vesicles are often supposed to be equivalent to or to be a modification of the endoplasmic recticulum. Oocytes often have a different kind of membranous structure. It takes the form of stacks of double membranes. Usually do not have

ribosomes attached to them but instead are performed by pores. These pores closely resemble the pores of the nuclear membrane.

Cortical Granules: In mature oocytes, formation of special structures known as the cortical granules takes place on the inner side of the plasma lemma. These are spherical bodies, surrounded by a simple membrane and containing acid mucopolysaccharides. The acid mucopolysaccharides of the cortical granules synthesize fertilization membrane during fertilization. The cortical granules are present in sea urchins, frogs, fishes, bivalve molluscks, some annelids, and some mammals (e.g., hamster, rabbit and man).

(ii) Vitellogenesis: Yolk appears in the oocytes in the second period of their growth called the vitellogenesis period. Yolk is the most usual form of food storage in the egg. In amphibians (Ward 1962; Baliksky and Devis, 1963) and fishes Yamamoto and Oota, 1967) the vitellogenesis or synthesis of different yolk components takes place inside modified mitochondria. In other vertebrates, the yolk is not synthesized in the oocytes at all but is produced in the liver of the body of the female, It is then transported in a soluble form via the blood stream and the follicle cells to the oocytes when it is finally deposited in the form of yolk platelets or yolk granules.

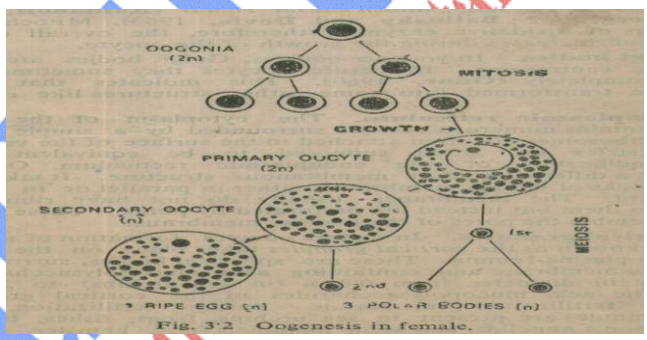


Fig. 3·2 Oogenesis in female.

C. Maturation Phase: The primary oocyte contains diploid number of chromosomes. After the oocyte complets its growth, it is ready for meiosis or reduction division during which the diploid chromosome number is reduced to haploid number. In this process, the primary oocyte is changed into haploid ovum or egg. This is called maturation. In spermatogenesis, the primary spermatocyte divides into four cells of equal size which eventually give rise to four sperms. In oogenesis only one ovum is produced out of the four unequal cells derived from the primary oocyte.

At the beginning of the maturation phase, the nuclear membrane breaks up and the contents of the nucleus get mixed up with the surrounding cytoplasm. The chromosomes which have become greatly contracted and concentrated toward the centre of the germinal vesicle, are carried to the periphery of the oocyte. An achromatic spindle is formed at the periphery, which takes up a position, perpendicular to the surface of the oocyte. The

bivalents come on the equatorial plate and subsequently separate into the two component chromosomes. Next, a bulge appears at the surface of the oocyte. The outer pole of the spindle with half of the chromosomes enters into this cytoplasmic bulge during anaphases first meiotic division. The bulge is then pinched off from the rest of the oocyte as a small cell, the first polar body. It receives only a very small quantity of cytoplasm, while the rest goes to the occyte which is now distinguished as the secondary oocyte. The secondary oocyte is of the same size as that of primary oocyte. Thus as a cones quence of the first meiotic division the primary oocyte divides into a large and a very small cell, each with a haploid number of chromosomes.

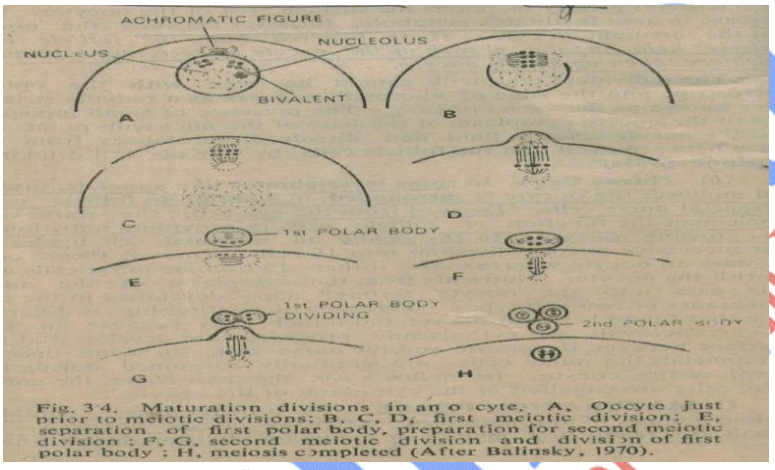


Fig. 3·4. Maturation divisions in an o cyte. A, Oocyte just prior to meiotic divisions; B, C, D, first meiotic division; E, separation of first polar body, preparation for second meiotic division ; F, G, second meiotic division and divisi on of first polar body ; H, meiosis c>mpleted (After Balinsky, 1970).

In the second meiotic division an achromatic spindle is again formed at the surface. When division takes place, half of the chromatids are given off, along with a small quantity of cytoplasm to form a secondary polar body. The larger cell, which receives the major part of the cytoplasm together with one half of the chromatids, represents the fully mature ovum. As the secondary oocyte is dividing, the first polar body divides into two cells. Therefore, in the maturation of egg, four cells are produced from one oocyte. One cell, the egg, as a functional gamete, the second and third cells are produced by the division of the first polar body, and the fourth cell is the second polar body. All the polar bodies disintegrate later because they have very little cytoplasm with no food reserves.

The unequal cytokinessis (cytoplasmic division) during oogenesis has the great significance for the egg. These unequal divisions allow one cell out of the 4 daughter cells to inherit most of the cytoplasm and reserve food material which is essential for the developing embryo.

4. Write short notes on the following-

(i) Acrosome formation

As the spermatozoa comes in contact with the egg, the acrosome of the sperm undergoes radical changes as the spermatozoa makes contact with the egg envelope, the sperm plasma membranes and the acrosomal membrane rupture at the point of contact.

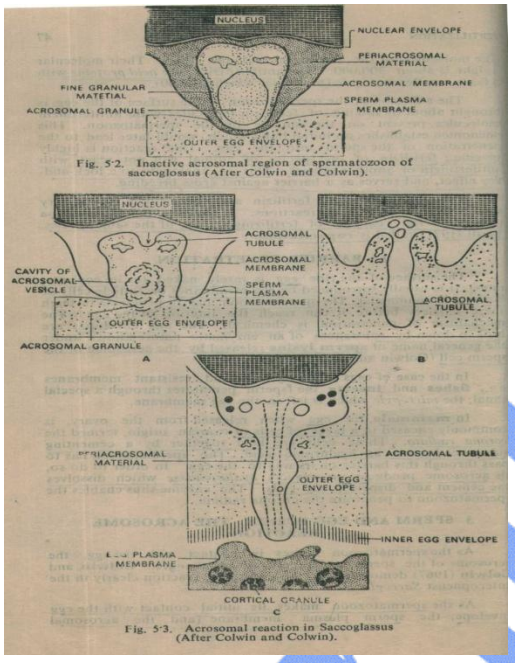


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Fig. 5·3. Acrosomal reaction in Saccoglassus (After Colwin and Colwin).

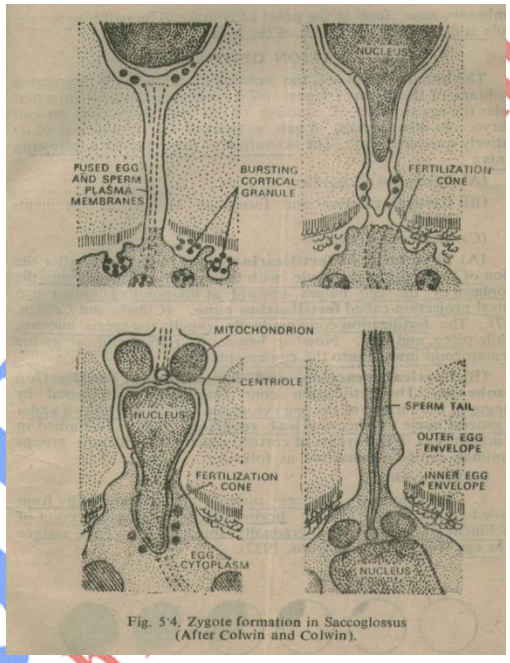

Fig. 5·4. Zygote formation in Saccoglossus (After Colwin and Colwin).

The acrosomal membrane then joins with the plasma membrane the margin of rupture so that the acrosomal granule is exposed to the surface of the egg envelope. Now the peripheral part of the acrosome collapses and its lysins (enzyme) are extruded. The central part of the acrosome elongates and becomes transformed into a long thin tube known as the acrosomal tubule. The acrosomal tubule traverses the egg envelopes (jelly and vitelline membrane) and finally touches the egg plasma membrane. These two membranes now fuse as the point of contact. The pathway for the tubule is cleared by the action of the lysins.

(ii) What effect does yolk take on cleavage?

Ans. Yolk is a mechanically inert material, which when present in large quantities, may interfere physically with the subdivisions of an egg during cleavage. It retards the progress of the furrow to divide the cytoplasm following nuclear division. During cleavage, the chromosomes and the achromatic spindle are generally shifted into the more protoplasmic portions and away from the yolky areas of the egg. Consequently, the protoplasmic portions divide into smaller cells than the yolky areas. Besides, they divide more frequently.

The cleaves mitosis. Mitosis is characterized by the movements of cell components viz., the chromosomes, achromatic figure, mitochondria etc. The activity of these components along the equator of the maternal cell leads to the ultimate separation of the daughter cell. The yolk granules or platelets are passively distributed between the daughter blastomers during these movements. When yolk become abundant, it tends to retard and the process of cleavage slows down. The yolk in the uncleaved egg is generally more concentrated toward the vegetal pole of the egg than the animal pole. Therefore, the cleavage is most retarded at the vegetal pole of the egg and here the blastomeres are larger in size.

Frog's egg is a good example to explain the effect of yolk on cleavage. The first cleavage furrow is meridional but it does not appear simultaneously all stround the circumference of the egg. At first it is seen only at the animal pole of the egg where the amount of yolk is less. It then gradually prolongs along the meridian of the egg. Passing through the yolk-laden cytoplasm or deutoplasm, it eventually reaches the vegetal pole. This divides the egg into two blastomeres. The same process is repeated during the second meridional cleavage which takes place at right angles to the first. During the third cleavage, when the plane of division is latitudinal, the cleavage furrow appears simultaneously all over the circumference of the egg because it meets with an equal resistance from yolk at all sites. There is a greater accumulation of yolk at the vegetal pole of telolecithal egg. This interferes with the cell division at this pole and as a result the cleavage here becomes inhibited.

The pattern of cleavage is determined considerably by the amount of yolk in the egg. Isolecithal and telolecithal eggs undergo complete or holoblastic cleavage. In this type, the cell membranes formed during cleavage cut completely through the egg. In centrolecithal and discoidal eggs, the cleavage is incomplete or meroblastic. Such eggs contain so much yolk that only a small amount of cytoplasm with nucleus undergoes segmentation.

Section – B

5. Describe various patterns and planes of cleavage.

During segmentation, the cleavage furrows are not formed at random.

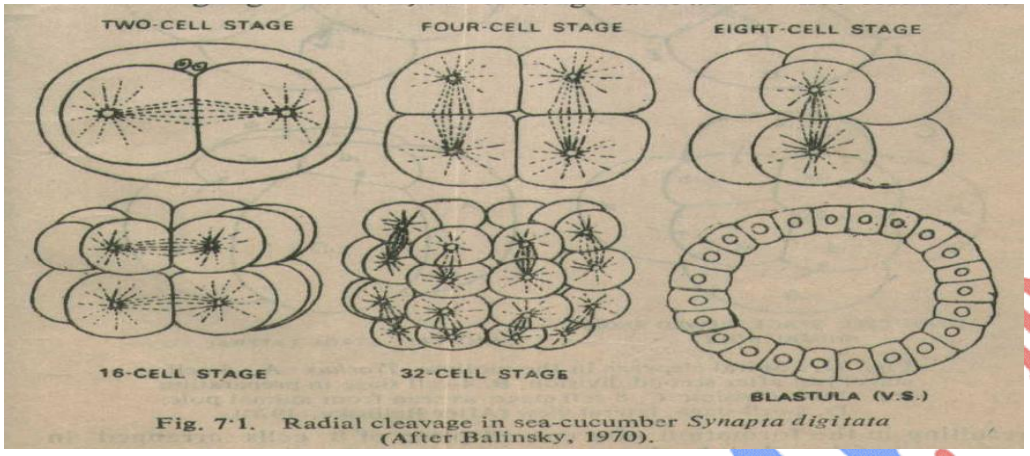

Fig. 7·1. Radial cleavage in sea-cucumber *Synapta digitata* (After Balinsky, 1970).

but are oriented in a particular with reference to the main (animal-vegetal) axis of the egg. The orientation successive cleavage furrows with respect to each other and to the main axis of the egg is, however, unlike in different species. As such, various patterns of cleavage are found among animals. Based upon symmetry, four patterns of cleavage have been recognized. They are radial, biradial, spiral and bilateral.

1. Radial Cleavage – In this cleavage pattern, divisions take place in such a manner that all the blasters are placed in a radically symmetrical fashions around the polar axis. When such an egg is viewed from the poles, the blasters seem to be arranged in a radically symmetrical form. Example: Sponge, coelenterates, sea urchin, sea cucumber, Amphioxus.
2. Biradial Cleavage- This pattern of cleavage is found in ctenophores like Beroe. Four blastomeres arise by the usual two meridional cleavages. The third

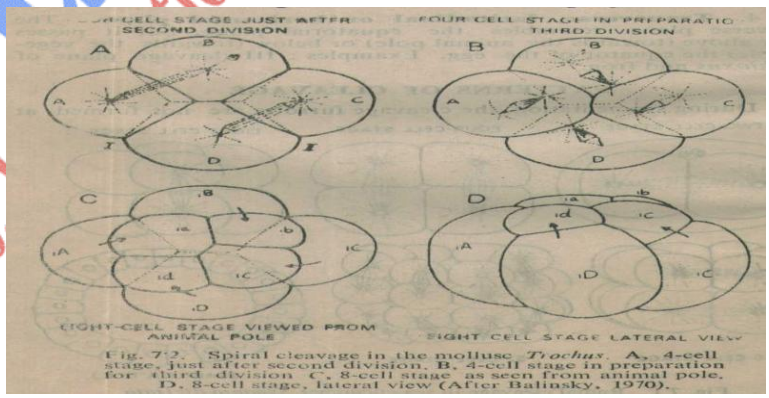

Fig. 7·2. Spiral cleavage in the mollusc *Trochus*. A, 4-cell stage, just after second division. B, 4-cell stage in preparation for third division. C, 8-cell stage as seen from animal pole. D, 8-cell stage, lateral view (After Balinsky, 1970).

Cleavage plane is vertical resulting in the formation of a curved plate of 8 cells arranged in two rows of a curved plate of 8 cells arranged in two rows of 4 each. In these rows, the central cells are larger than the end ones.

3. Spiral Cleavage – The spiral cleavage is diagonala to the polar-axis. In this type, the spindles for the third cleavage, instead of being erect, are oriented diagonally so that the rsuolting upper tier of cells is displayed sidewise. The ;upper 4 cells are placed over the junctions between the four lower cells. The upper smaller cells are called lmicro-, and lower larger cells are known as micrometers. The spiral cleavage results due to oblique positions of the mitotic spindles. This type of cleavage is called the spiral type because the four spindles during the third cleavage are arranged in a sort of spiral.

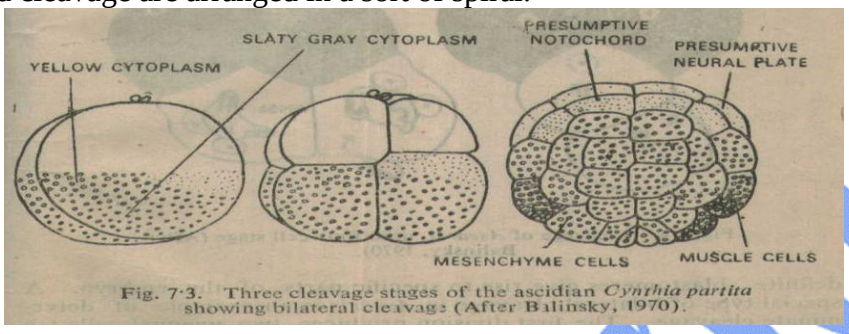

Fig. 7·3. Three cleavage stages of the ascidian *Cynthia partita* showing bilateral cleavage (After Balinsky, 1970).

The turn of the spiral as seen from the animal pole may be in a clockwise direction (dextral) or in a counterclockwise direction (sinistral). Moreover, the spindles are alternatively titled obliquely to the dextral and sinistsral directions, so that successive generations of blarometres are oriented in a twisted fashion.

The pattern of cleavage characterizes the egg of annelids, mollusks, nemerteans and some of the planarians.

4. Bilateral Cleavage – In this pattern cleavage, the blasters are so arranged that the right and left sides becomes distinct. This cleavage pattern is dependent upon differences in the size of the blasters. In this case, two of the first four blastomeres may be larger than the other two, thus establishing a plane of bilateral symmetry in the developing embryo. Subsequent cleavages may make the bilateral arrangement of the blasters still more obvious. Examples are nematodes, cephalopod molasses, some echinoderms and tunicates.

5. The cleavage is initiated by the appearance of a constriction of groove called cleavage furrow. The cleavage furrows may divide the egg from different angles of planes. There are four important planes of cleavage.

1. meridional plane: When cleavage furrow bisects both the poles of the egg, passing through the animal-vegetal axis, the plane of cleavage is called maridional plane. Examples are, I and II cleavage furrows in frog; I cleavage furrow in chick.

2. Vertical plane: A furrow which passes from the animal pole to the vegetal pole, but it does not pass through the median axis of the egg. Instead, it is oriented towards one side of the axis. Example: III cleavage furrows in *Amia calva*, *Lepidosteus osseus* and chick.

3. Equatorial plane: This plane of cleavage bisects the egg at right angles to the median axis and half way between the animal and vegetal poles. Example: I cleavage plane of eggs of higher mammals.

4. Transverse, Latitudinal or horizontal plane: The transverse plane resembles the equatorial plane, but it passes either above (towards the animal pole) or below (towards the vegetal pole)

6. The equator the egg. Examples: III cleavage plane of Amphioxus and frog.

6. Write short notes on

(i) Blastulation

Ans. The development of blastula is called blastulation. The types of blastulae greatly varies among different animals depending upon various factors such as the egg size, the amount and distribution of yolk, and the rate and pattern of cleavage. The various types of blastulae can be classified into four types: (i) stereoblastula, (ii) Coeloblastula, (iii) periblastula, and (iv) discoblastula.

(1) Holoblastically Formed Blastulae: In holoblastic embryos, the blastula is either solid (stereoblastula) or hollow coeloblastula).

(i) Steroblastula. This type of blastula is composed of densely-packed but larger sized and relatively small number of cells. Blastocoelic space in the centre is very small or virtually absent. The blastomeres in a stereoblastula may reach from the surface to the centre; or separate blastomeres may occupy the interior. Examples; Insects, some worms (e.g., Nereis) and some mollusks (e.g., Crepidula).

(ii) oeloblastula. This type of blastula consist of one or several layers of cells arranged around a large blastocoel. It is of two types:

(a) Adequal. The cells of the blastoderm of about the same size throughout. The blastocoel is centrally placed, e.g., echinoderms.

(b) Inequal: The wall of the vegetal half is thicker than that of the animal half. The blastocoel is reduced in volume and distinctly eccentric, i.e., displaced towards the animal pole, e.g., Amphioxus, amphibians.

(ii) Egg membranes

Ans.

1. Yolk Sac

Development: In reptiles and birds the someatopleure and splanchnopleure develop from the periphery of the blasto disc which is differentiated as the area opaca. These gradually spread peripherally over the yolk mass. Soon afterwards, the embryo begins to be undercut by a series of folds which appear all around the body of the embryo. The folds involve all three germ layers, i.e., the ectoderm, the mesoderm and the endoderm. Directed downward and inward, these are known as the body folds.

The extra embryonic splanchnopleure (splanchnic mesoderm+ Endoderm) continues to spread over the yolk mass and as a yolk sac eventually encloses the mass of yolk in a large measure. The yolk sac, however, never surrounds the yolk fully. A small passage is left on the ventral side for the embryo to absorb the remains of albumen at a later stage.

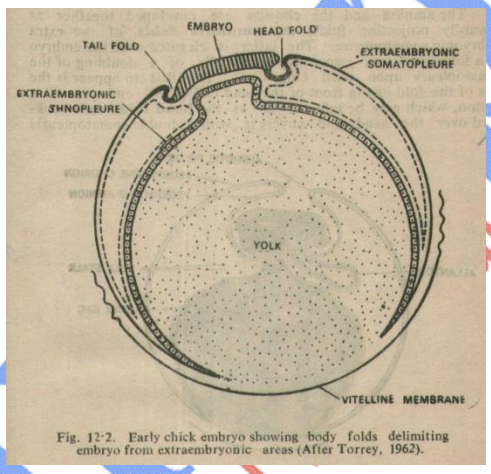

Fig. 12·2. Early chick embryo showing body folds delimiting embryo from extraembryonic areas (After Torrey, 1962).

Coincidently with the formation of the yolk sac, the intra embryonic splanchnopleure is subjected to folds resembling with the more superficial body folds. The intra-embryonic folds give rise to a walled digestive tract, or gut, in the body of the embryo. The middle of the embryonic gut remains open to the yolk beneath. At this level the yolk sac is connected to the digestive tract by a constricted yolk stalk.

The wall of the yolk sac is thrown into folds on its innor surface that pentrate the yols mass. These folds are known as the side yolk sac septa. A system of blood vessels is developed in the walls of the yolk sac, entering the heart by means of a pair of viteline veins.

Functions:

The function of yolk sac is to digest the yolk and to transfer the products of digestion to the embryo. Digestion is brought about through the mediation of appropriate enzymes secreted by the endodermic cell lying in contact with the yolk, particularly at the yolk sac septa.

Although the yolk sac is connected to the digestive tract by the yolk stalk, the yolky food is not transported to the embryo by this route. Instead, the products of yolk digestion are picked up and carried to the embryo by the Vitelline veins running in the mesoderm of the sac.

2. Amnion and Chorion

The amnion and the chorion are developed together as upwardly projecting folds, the amniotic folds of the extra embryonic somatopleure. The latter is elevated over the embryo by a folding process consisting essentially of a doubling of the somatopleure upon itself. The first to appear is the part of the fold just in front of the head end of the embryo. This section, which may be referred to as the head fold bends backward over the head and covers it with a double somatopleuric hood. Now the lateral ends of the fold are prolonged backward along both sides of the embryo. The lateral folds come near each other over the body of the embryo and fuse from the front end backward, so that more and more of the embryo becomes covered by the folds. Eventually, a tail fold also develops behind the embryo. All these folds finally converge so as to encase the embryo in two sheets of somatopleure. The inner somatopleuric sheet becomes the amnion, the outer, and the chorion. The point where the head and tail folds meet is called the sera-amniotic connection. It lies somewhat behind the middle of the embryos body.

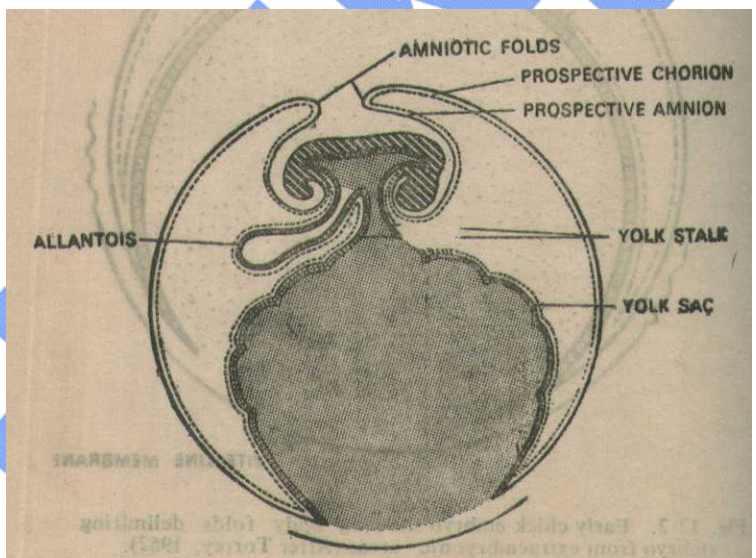

Fig. 12·3. Early stage in the development of the extraembryonic membranes of the chick (After Torry, 196).

The amnion consists of a layer of extraembryonic ectoderm on the inside and a layer of extra embryonic somatic mesoderm on the outside. The chorion is made up of a layer of extra embryonic ectoderm on the outside and a layer of extra embryonic somatic mesoderm on the inside. The latter is however, continuous with the ectoderm and splanchnic mesoderm covering the yolk sac. The cavity between the amnion and embryo is termed the amniotic cavity. In between the amnion and the chorine cavity or extra embryonic coelom, which is

continuous with the coelom, which is continuous with the coelomic cavity in the embryo proper.

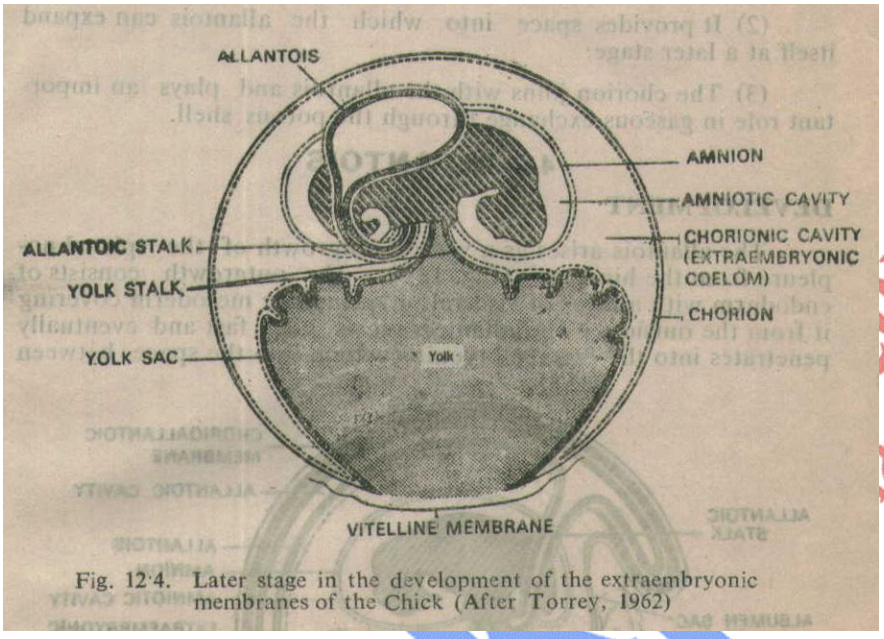

Fig. 12·4. Later stage in the development of the extraembryonic membranes of the Chick (After Torrey, 1962)

Function of Anmion

1. The primary function of the amnion is to protect he embryo from desiccation. The embryo gets a special advantage by the development of a fluid-filled amniotic cavity. It becomes immersed in a container filled with fluid and thus can carry out its development in a fluid mecum, although the egg is placed on "dry land".
2. Muscle fibers differentiate within the mesoderm of the amnion. The amnion with its muscle fibers and contained fluid serves as an efficient shock absorber. It protects the embryo from mechanical shock resulting from the possible violent agitations of the whole egg caused by incubating hen sitting on it.
3. The amnion separates the embryo from the shell of the egg. Thus it protects it from adhesion to the shell or from friction against it.

Function of Chorion

1. The fluid of the chronic cavity, which is also called the extra-embryonic coelom, provides further protection to the embryo.
2. It provides space into which the allantoises can expand itself at a later stage.
3. The chorion joins with the allantois and plays an important role I gaseous exchange through the porous shell.

4. Allantois

The allantois arises as a ventral outgrowth of the splanchnopleure from the hindgut. (Fig.) the outgrowth consists of endoderm with a layer of visceral or splanchnic mesoderm covering it from the outside. The allantois grows very fast and eventually penetrates into the extra-embryonic coelom, into the extra-embryonic coelom, into the space between the yolk sac, the amnion and the chorine. The proximal part of the allantois forms a narrow neck or the allantoic stalk with which it remains connected with the hindgut of the embryo.

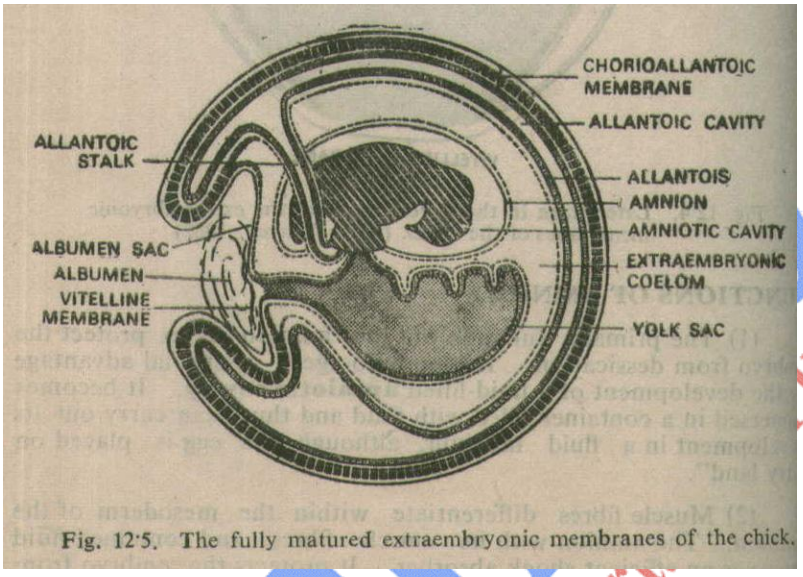

Fig. 12·5. The fully matured extraembryonic membranes of the chick.

The distal part of the allantois expands and penetrates between the anminon and the yolk sac on one side and the chorion on the other side. By the middle of the incubation period, the allantois spreads all around the egg underneath the chorine.

The mesoderm on the external surface of the external surface of the allantoises fuses with that of the chorion forming a conjoined chorioallantoic membrane. Actually, it consists of three layers:

(i) Ectoderm on the outside.
(ii) Mesoderm (splanchnic layer of allantois +somatic layer of chorion) in the middle.
(iii) Endoderm on the inside.
(iv) The chorioallantoic membrane gets pressed against the porous shell. It becomes highly vascular by developing allantois circulation.

Functions

(i) The cavity of the allantois serves as a urinary bladder. It stores the protein breakdown products in the form of water-insoluble crystals of urinc acid inside the egg up to the time of incubation, the allantois increases to enormous proportions.
(ii) The vascular "choriaoallantoic membrane lies in a close proximity to the inner surface of the porous shell. It acts as an extra-embryonic lung by supplying the embryo with oxygen. Gaseous exchange takes place between the blood circulating in the chorioallantoic membrane and the external air through the porous shell. A

network of blood vessels develops in this membrane and this network is in communication with the embryo proper by means of blood vessels running along the allantois stalk. The allantois circulation is continued till the chick breaks the egg shell and begins to breathe the air.

(iii) Together with the chorion, the allantois also surrounds the albumen to form the albumen sac and thus assists in the absorption of nutritionally rich albumen.

7. Write short notes on

(i) Introduce the nature of induction

Ans. Soon after Spemann's discovery, it was found that the inducing properties of the organizer (dorsal lip of the blastopore) were retained even if the cells were killed by various means by boiling, by treating with alcohol or petrol ether, by freezing or by dessication. When implanted into a living embryo, it was found that a killed organizer can still induce (Bautzmann, Spermann and Mangold 1932). It indicated that the vital activity of the organizer is not essential for induction. Probably, the roof of the archenteron produces its effect by liberating some chemical substance which is the immediate causes of induction. It is acceptable that such a substance could be liberated even from dead tissues. Waddington, Needham and Coworkers concluded that this natural inducing substance is a steroid. Arey (1966) called it an evocator.

Abnormal inductors and induction by substances of known chemical substance. It has been found that not only the chordo-mesoderm but also a wide variety of tissues like liver, kidney, muscles, gut and skin etc. are able to produce induction. Induction can also be performed by tissues of various animals belonging to different phyla of the animals kingdom. For example, the tissues of Hydra, insects, fishes, reptiles, birds and mammals were found to be effective as inductors of newt. Neural inductions may result from such an unlikely agents as methylene blue or a mechanical irritant such as sillicious earth. Some worker have presented evidence that ribonucleoprotein, i.e., a complex of RNA and protein may have inductive properties. Besides, a number of weak organic acids are good inductors. These include muscle adenylic acid (AMP), thymonucleic acid (DNA), dithydroxystearic acid, linolenic acid and stearic acid.

(ii) Morphogenic movements

Ans. The events which transform a blastula into a gastrula are collectively called gastrulation. According to MeEwen (1923) gastrulation fefers to the formation of the primordial gastric or gut cavity, the process also involves the development of two of the

three primoridial germ layers, referred to as the ectoderm (epiblast) and endoderm (hypoblast). The third layer called mesoderm (mesoblast) is derived from one of the other or both of the two layers; the endoderm is the inner layer; and the mesoderm is the middle layer.

Lankester (1875) and Hubrecht(1906) were of the opinion that the gastrulation is a process during which the single layered blastula is converted into a two-layered (e.g., Amphioxus) or three- layered (e.g., most vertebrates) embryonic stage called gastrula.

During gastrulation, the cells are reorganized and rearranged. This arrangement of cells is brought about by very complex but co-ordinated movements and shifting of positions of large masses of cells. According to Balinsky (1970) "The process of gastrulation involves involves displacement of parts of the early embryo. As a result the endodermal and mesodermal organ rudiments are removed from the surface of the embryo, where the presumptive material for these rudiments is to be bound in the blastula stage. These are then brought into the interior of the embryo, where the respective organs are found in the differentiated animal. Concomitantly, the single layer of cells, called blastoderm, gives rise to three germinal layers- the ectocderm, the endoderm and the mesoderm".

The Prominent Features of Gastrulation

1. Arearrangement of the cells of the embryo takes place by means of formative or morphogenetic movements.

2. The pace of cellular division is slowed down.

3. Growth, if any, is trifling.

4. The rate of oxidation is intensified and the type of metabolism changes.

5. The nuclei become more active in governing the activities of the embryonic cells.

6. The influence of the paternal chromosomes becomes clear during gastrulation.

7. New kinds of proteins, that were formerly not present in the egg begin to be synthesized.

Section – C

8. Describe the types of placentae in mammal.

A Mammalian placenta typically is a structure produced by the apposition or fusion of the extra embryonic membranes with the endometrium of uterus for the purpose of physiological exchange. It, therefore, follows that the placenta from the point of view of its origin, consists of to parts a foetal placenta, furnished by the extra embryonic membranes and a maternal placenta, furnished by the uterine endometrium. Usually, the trophoblast cells of mammalian embryo remain specialized for interaction with the uterus.

Now it would be obvious that while on the material side a single component, the endometrium is involved; on the foetal side chorium yolk sac and allantois. The first of these, the amnion, may be ruled out immediately, as it is making no direct contribution to the placenta. This laves the other three, of which the chorion, because of its most extermal position, its membrane making immediate contact with the endometrium. But, we have seen in chick embryo that the chorion plays its role by way of a vascular supply, which it acquires from the allantois. In mammals, there are two possible sources of chorionic vascularizaiton- the vitelline circulation provided by the yolk sac and allantoic circulation provided by the allantois. Thus, it can be said that in mammals, there exist two essentially different main types of placenta, the chorio-vitelline placenta and the chorio-allantoic placentas.

A. Chorio-vitellineplacenta

In some mammals notably some marsupials (Didelphys Macropus) the allantois remains relatively small and never makes contact with the chorion, whereas the yolk sac becomes very large nad tets fused broadly with the chorion. In these forms the chorion gains its blood supply from the network of vitelline blood vessels of yolk sac such a placenta is called yolk sac placenta or chorio vitelline placenta. In such a foetal placenta the chorion never advances beyond a smooth membrane in close apposition with the vascular uterine lining the endometrium.

B. Choio-Allantoicplacenta

In some marsupial (e.g. Caramels, Dasyures), and all Lutherans the yolk sac remains rudimentary and the allantoids becomes well developed and vascularized to fuse with chorion and to furnish the latter the blood supply.

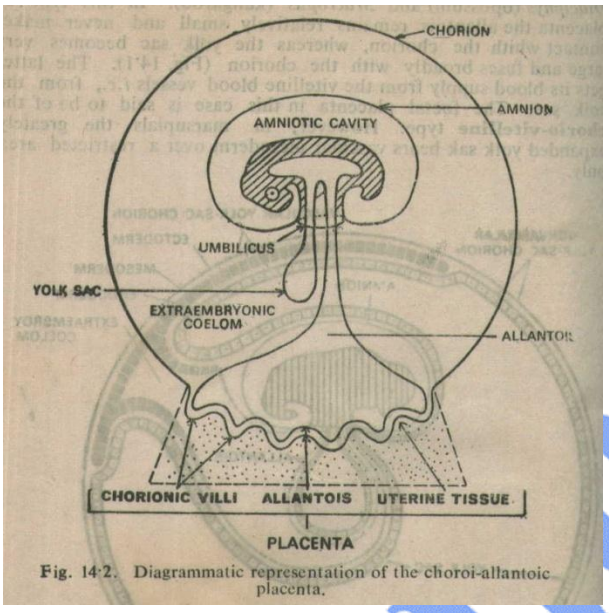

Fig. 14·2. Diagrammatic representation of the choroi-allantoic placenta.

Such a foetal placenta is called chorioallantoic placenta. In this kind of placenta, the chorion is not smooth but bears root-like vascular processes, the villi, which grows out from the chorion into the adjacent maternal tissue. The chorioallantoic placenta originates in the following fashion.

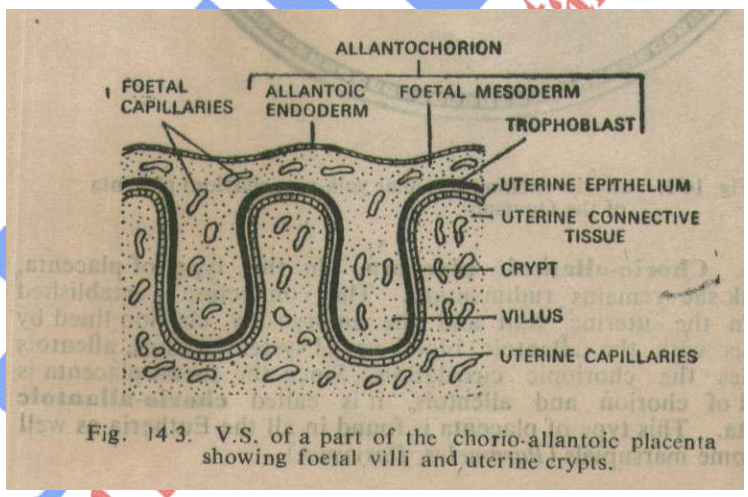

Fig. 14·3. V.S. of a part of the chorio-allantoic placenta showing foetal villi and uterine crypts.

Development of Chorio-allantoicplacenta

When a mammalian embryo enters the uterus, the zona pellucid, which previously surrounded it, becomes dissolved and the embryo (blastocyst) is bathed by the uterine fluid. The fluid contains organic substances produced by the tubular glands of the uterine wall and voided into the lumen of the uterus. The early embryo may absord some of these substances through its epithelium covering so long as a closer connection with the uterine, wall has not been established. For its further development however, the embryo is completely dependent of substances supplied to it from the tissues and the maternal

tissues is essential. Nevertheless, it is found that the closeness of this connection between foetal and maternal tissues differs greatly within euphoria mammals. On the degree of intimacy of foetal and maternal tissues following three types of placenta may be recognized;

1. Non- decideuous placenta or semi placenta – In most mammals, the implantation is superficial, i.e. the blast cyst lies in the cavity of the uterus in contact with the uterine wall. The ocontact may be made more intimate by the surface of the blastocyst by forming finger-like outgrowth which penetrate into depression ins the wall of the uterus. Such outgrowths are initially formed by the trophoblast (i.e. the epithelial layer covering the blast ocyst), but later on the connective tissue and blood vessels invade the outgrowths. These outgrowths are called chorionic villi, the blood vessels of Chorionic Villi are the branches of Allantoic bllod vessels in case of chorioallantoic placenta. (In chorio-vitelien placenta, viteline blood vessels give their branches to chorionic Villi).

At the time of birth, when parturition (the separation of the foetus and its membranes from the mother's body) occurs, the chorionic Villi are simply drawn out from the depressions in the wall of the uterus, and thus maternal and foetal tissue are separated without further damage to the uterine wall and no bleeding occurs. This type of placenta is called non-deciduate or non- placenta deciduous placenta and is found in pigs, cattle and some other mammals. Further, the chorionic villi of a non-deciduate placenta, because lie in apposition with the endometrium, but do not fuse with it, so such a placenta is also called semiplacenta.

2. Deciduous placenta or placenta Vera- In other mammals, however, the degree of intimacy between maternal and foetal tissues becomes further increased. The wall of the uterus becomes eroded to various degrees through the action of the trophoblast and the embryonic tissues penetrate into the uterine wall establishing a more intimate contact and facilitating the passage of substances from the mother to the foetus and from the foetus to the mother. Here because eth chorionic villi fuses with the eroded uterine mucosa, such placenta is called placenta Vera (true placenta). At the end of pregnancy the uterine walls is no longer intact and when the fuetus with its membranes including the chorion is removed, more or less extensive hemorrhage from the uterine wall ensues (i.e. at birth, when placenta is discharged, the uterine lining also tears away with some bleeding. Such a type of placenta found in higher eutherian mammals is called deciduate or deciduous placenta.

The maternal tissues which are expelled at birth in the case of deciduate placenta are called deciduae. These are three regions: (i) The part which lies between the chorionic vesicle and the muscles of the uterus wall is the deciduas basalis (ii) The part which surrounds the Chorionic sac and separates it from the cavity of the uterus is the

deciduas capasularies. (iii) The part which forms the inner lining of the rest of the uterus is decidua parietalis.

The hemorrhage at parturition is normally stopped by the same mechanism as serves for the expulsion of the newborn, the contraction of the muscular wall of the uterus constricts the blood vessels and thus, slows down the flow of blood, until clotting of the blood stops the hemorrhage altogether.

3. Contra-deciduate placenta- In paameles and Talpa (male), somewhat modified type of deciduate placenta occurs, which is called contra-de-ciduate placenta. In such case, not only there is a loss of maternal tissue but also of the foetal portion of the placenta, both of which absorbed in situ by maternal leucocytes.

9. Write notes on the following-

(i) Stem cells

Ans. Application

1. Current research on stem cells is focused on how stem cells differentiate into specific types of cells. The researchers' goal is to figure out what genetic and environmental signals trigger the stem cell to develop into a specific type of cell. We need to determine which factors "tell" the cell to divide or specialize. Studying the development of normal cells and tissues will lead to a better understanding of abnormal growth and development, which could lead to new ways to prevent birth defects and cancer.
2. Stem cells could be used in testing medicines. Pharmaceutical companies currently rely on test subjects to see the effects of medicines. The use of stem cells would enable scientists to culture more and more human cells for testing purposes, to study the good and bad effects of a drug on a particular type cell. This would potentially reduce the numbers of animal and human testing.
3. Stem cells can be used to cure diseases caused by the premature death of cells. Other researchers hope to utilize them to produce replacement of human organs.
4. It is also possible that stem cell research may hold the key to slowing down the ageing process.

Role of Stem Cells in Diseases Caused by Cell Failure

(a) Heart damage: The heart's cardiac muscle, do not regenerate by itself, so a damaged heart stop working. There is hope that the stem cell therapy can repair heart muscles and save lives. Stem cell therapy for heart disease is envisioned as having generic stem cells grown into cardiac muscle cells. These cells would then be injected into the heart, where they would grow and reinforce the existing heart tissue. Experiments shown that transplanted heart muscle cells successfully augment and integrate with

the host cells. The unfortunate few who get the disease now may be able to rely on stem cell therapy.

(b) Myocarditis results in inflammation or degeneration of the heart muscle. Although it is often caused by other diseases, it can occur as a stand-alone disease in adults or as a result of ageing. Heart attacks occur when not enough oxygen reaches the cardiac muscle. Cardiac muscle requires a constant supply of oxygen; it will die rapidly if blood stops purpling through the body. The heart is thus weakened, and may not be able to pump as effectively. Regenerating cardiac tissue is what researchers hope to achieve. In cases of extreme heart damage or deformation, a heart transplant may be necessary. With stem cell technology, scientists may be able to grow heart form a patient's own stem cells. Heart transplants today are high risk because the receiver's immune system may see the donor organ as foreign and attempt to destroy it. Recipients usually must take anti rejection drugs for the rest of their lives to retain the organ. Cloned hearts, since they are from the same person, would not be at risk of rejection.

(c) Diabetes: Diabetes is a disease where the production of insulin is disrupted, or where the insulin produced by the body is defective. Diabetics must periodically inject themselves with insulin to ensure that they get enough glucose. In type I diabetes, the production of insulin is disrupted in some way. The specialized cells, called islet cells, do not produce the necessary insulin. There is research that suggests transplantation of either the entire pancreas of isolated islet cells could reduce or do away with the need for insulin injection. The treatment for diabetes would use stem cells to make islet cells for diabetes research and then hopefully transplantation. Sufferers of diabetes can look forward to a needle free future.

(d) Treating Other Diseases: Diseases such as cancer are different in that they are malignant growths of cells. Scientists will be able to use the knowledge gained from studying stem cells to treat cancer, potentially by using chemical signaling to tell the cancerous cells to stop dividing. The growth of the tumor could then be halted, and if another growth appeared, the same approach could be used. Studies on cancer cells have shown that some types strongly resemble stem cells, and studies on these cells may prove to be useful.

Treating Cell Failure: Spinal cord injuries, Parkinson's disease, leukemia, AIDS and Alzheimer's disease all kill cells. The detrimental effects of these diseases stem from the loss of critical cells. In spinal cord injuries, Parkinson's disease, Huntington's disease, and Alzheimer's disease, neurons are lost or damaged. This most often results in paralysis and/or loss of control of the motor system.

A recent procedure conducted on a person with Parkinson's used pig stem cells. They were injected into his brain, and had an amazing effect, improving his motor skills drastically. This procedure has been conducted on many people, and most have shown a positive response to the therapy. The procedure could potentially be used on patients with other types neurological disorders.

New research has shown that there are often reserves of stem cells left inactivated throughout the body. If scientists can figure out how the activate these reserves, no

injections of cells will be needed for treatment. The AIDS virus attacks the CD-4 immune cells, essential disease-fighting cells. Using stem technology, scientists may be able to make these cells and bolster a patient's immune system.

(ii) Nuclear transfer technique.

Ans. Each cell of living organisms contains a "blueprint" for its development. These instructions are composed of DNA which is located in the nucleus of cell. Soon after egg has been fertilized the cells of the developing embryo begin to acquire specialized functions, becoming for instance, brain cells or skin cells. This is called cell differentiation. Once a cell has become differentiated, it never reverts to the prior undifferentiated state.

Somatic Cell Nuclear Transfer (SCNT) Method: First explored by Hans Spemann in the 1920's to conduct genetics research, nuclear transfer is the technique currently used in the cloning of adult animals. All cloning experiments of adult mammals have used a variation of nuclear transfer.

A somatic cell is any cell other than a sperm, egg, or cell that gives rise to a sperm or egg. Nuclear transfer requires two cells, a donor cell and an acolyte, or egg cell. The nucleus of the egg (containing its DNA) is removed and replace with the nucleus (and its DNA) of a somatic cell (such as skin of blood) from the recipient. Research has proven that the egg cell works best if it is unfertilized, because it is more likely to accept, the donor nucleus as its own. The egg cell must be enucleated, which eliminates the majority of its genetic information. The donor cells is then forced into the Gap Zero, or Go cell stage, a dormant phase, which causes the cell to shut down but not die. In this state, the nucleus is ready to be accepted by the egg cell. The donor cell's nucleus is then placed inside the egg cell, either through cell fusion or transplantation. The egg cells are then prompted to begin forming an embryo. (To harvest stem cells, the egg containing the transferred nucleus is encouraged to divide until it reached the blast ocyst stage, at which time the cell of the inner cell mass are removed and cultured. These are known as embryonic stem cells or ESP's). The embryo is transplanted into a surrogate mother if stem cell is not the goal. If all is done correctly, occasionally a perfect replica of the donor will be born.

1. The roslin Technique- The Roslin Technique for cloning (so named because it was developed at the Roslin Institute in Roslin; Scotland) was pioneered by IanWilmot and Keith Cambell. The technique is aimed at synchronizing the cells cycle of the donor and egg cells. To synchronize the cells, the donor cell is essentially "starved," only receiving enough nutrients to keep it alive. This is done to make the donor cell go into a suspended, quiescent state, known as the Go stage. Next, the enucleated egg cell is placed next to the donor cell. After that, an electrical pulse is fired to fuse the donor and enucleated egg

cells together and activates the embryo. Any surviving embryo is placed into the uterus of a surrogate mother ewe. If all has gone, well, a cloned sheep will develop.

The invention is covered by two patent application fields by roslin institute (Edinburgh) with a priority date of 31 august 1995: pct/gb96/02099, entitled –quiescent cell populations for nuclear transfer and pct/gb96/0298, entitled-inactivated oocytes as cytoplast recipients for nuclear transfer. These application cover use in all animals and in most countries of the world.

Creation of dolly using nuclear transfer technique:

In the 1980s, scientists cloned a sheep embryo using a nuclear transfer technique. Two groups of cells were used in this process one group of unfertilized eggs and one group of undifferentiated embryonic cells. The nuclei were removed from the unfertilized eggs (stripping them of DNA) and replace with undifferentiated embryonic cells. This was done to transfer the embryos' DNA into the unfertilized egg cells. The embryonic clones created from this technique were then implanted in the uteruses of genetically unrelated sheep for the remainder of their gestation.

An elaboration of the nuclear technique allows the cloning of mature organisms. This is done by placing the differentiated cells of the donor organism in a culture that cause them to return to their undifferentiated state before proceeding with nuclear transfer. "Dolly," a sheep produced in Scotland, was the first successful clone on an adult animal. The production of Dolly was significant because this meant an animal with known properties, such as rate of growth and milk production could be cloned, whereas the productivity traits of offspring cloned from embryos remain unknown until these animals mature.

The most popular clone, Dolly the lamb, was created using the Nuclear Transfer Technique. The first process of this technique is to extract and culture embryos, causing them to produce multiple genetically identical individuals. Several months later, when the embryos, also known as donor cells, are ready for cell transfer, the egg, or recipient cell, must be prepared. Chromosomes (one of the primary genetic components of a species) are removed from the egg with laser precision using a process known a micromanipulation. The donor cell also must be prepared. It must "forget that it was specialized and return to a non-specialized, embryonic state", Placing the cell in a salt solution deprives it of necessary nutrients, causing the cell to "forget its specialization." When an egg is first formed and begins to grow, its cells are non-specific and only later learn to grow into specialized body parts like arms or organs. By "non-specializing" the donor cell, it will create an entire organism, instead of the specialized organ in eventually became. In addition the donor cell will not be rejected by the egg during nuclear transfer. The donor cells are the ready to be fused with the egg. Originally, scientists believed the nuclear transfer technique would not likely be applied to humans because the genetic

composition of human is much more complex then of a sheep. Because of the human cell's more complex DNA structure, placing a human egg cell in salt solution does not have the same "non-specializing" effect of animals cells New research suggests that the Nuclear Transfer method could be used after all. A clone's genetics produced by the nuclear Transfer method technique are identical to those of the donor, and completely independent of the recipient egg cell. Therefore, any egg cell, such as a cow, can be used to produce clones of different species just by implanting a donor cell from the desired species into the cow egg cell.

A research company known as ABC Global has taken new steps in the method by using a cow egg cell for multiple species. The cow egg cell is used because it is larger, and therefore, cheaper and easier to obtain for research purposes. The Nuclear transfer technique with recipient cow egg cell has already been used to produce sheep, monkeys, and pigs from donor cells taken from the ears of these animals. Between 7-9 days after the donor cell is implanted in the cow egg cell and the cells are electrically shocked, the combined cell is implanted into the uterus of a surrogate mother of the same species as the donor.

This new research strikes fear into the opposition to human cloning. The opposition was quelled by the assurance that the technological era could not produce a clone in the near future because of the difficulty to extract enough human cells to finally succeed in the process. (Dolly required 277 trails before success, and the complexity of the human cell suggested that the number of trials required to clone a human cell would be much greater). The ability to use cow egg cells has greatly increased the chances of a human clone in the near future. David Magnus, an ethicist and researcher at the Center for Bioethics at the University of Pennsylvania, stated, "Cloning experts have been saying we had some lead time before we had to worry cloning human beings, but because cow eggs can be harvested cheaply and easily, one crucial barrier to human cloning may have fallen".

2. The Honolulu Technique: The Honolulu Technique was developed at the University, of Hawaii, by Teruhiko wakayama and Ryuzo.

Yanagimachi- It significantly differs from the Roslin Technique in that if does not use the risky procedure of an electric charge for cell fusion. Instead, the donor cell's nucleus is transferred to an enucleated egg and then allowed to sit for an hour, after which it is treated in a chemical bath containing strontium and cytochalism B to activate the cell for five hours. Also, the Honolulu technique used three types of donor, cells, all of which are usually in or are close to the Go stage.

Sertoli, neuronal, and cumulus cells – These Scientists discovered that a relatively high proportion of the oocytes developed into blast ocysts and then further developed when we

included a delay, He estimated that out of every 100 blastocyts transferred to wombs, seventy –one were able to take, from which between five and sixteen fetuses developed, and eventually two or three live mice were born. The Honolulu technique's high success rate (in comparison to the roslin technique) is a promising development in cloning research.

10. Discuss the following-

(i) Effects of Ageing

Ans. Particular efforts are being made to learn more about possible life prolongation and more attention is being given to life extension science. Premature ageing diseases (Progeria, Werner syndrome, Senescent accelerated mice), animals genetically modified by insertion or inactivation of genes (transgenic animals) and in vitro cell cultures allow identification of several mechanisms involved in life extension. Increased capacity to resist oxidative stress is particularly important.

The in balance in the dietary supply of sugars, proteins, and lipids may initiate major health problems including obesity, coronary heart disease, cancer, diabetes mellitus, high blood pressure, stroke, gout, and gall bladder disease, in old people a lack of vitamins causes vitamin deficiency. Antioxidants are natural substances that may help to prevent ageing –related diseases.

With age, the efficiency of antioxidant enzymes declines and ROS induced damage increases. To compensate for antioxidant enzyme failure, food supply in antioxidant is necessary. The best way to get anti-oxidants is by eating fruit and vegetables rather than by taking vitamin pills but more research is needed before specific recommendations can be made:

Hormone replacement therapy:

Hormone replacement therapy (HRT) can ease symptoms of menopause and protect against risk of heart disease, stroke, and osteoporosis.

However, all treatments related to hormone replacement should be undertaken with caution since several side effects may be observed.

Memory loss prevention:

Memory loss is not inevitable. Some simple devises may help to keep the memory intact: Writing things down, always putting frequently used items in the same place, repeating information that one needs to remember over and over again, making associations and relying on situation to rigger memory (for example, leaving an umbrella by the door).

Physical exercise:

Physical exercise is essential for a successful ageing. It helps to keep cardiovascular fitness reduces risks of osteoporosis and increases the sense of equilibrium.

(ii) Xenobiotics

Ans. Xenobiotics are biologically active substances that are "foreign to a given species. The most practical example is pesticides. For many pesticides to be effective, they must enter the phloem. Foliar-applied substances may diffuse into leaf through cuticle or enter stomata.

Movement through the plasmalemma is dependent upon polarity/hydrophobic. This is why pesticide formulations are so important. The body is exposed to foreign chemicals from many sources. Studies have shown that the "total load" of toxins to which the body is exposed is inversely related to its ability to detoxify them. Increased exposure to xenobiotics and antitoxins results in decreased detoxication capacity. Reducing exposure to toxin helps not only by decreasing toxicity directly but also by increasing the body's ability to defend itself against remaining toxins. Lifestyle, environment and dietary factors play a significant role in determining the body's total load of toxins. For example, Sources and distribution of xenobiotics. Large quantities of toxic chemicals are produced each year from industrial sources. Many of these pollutants are introduced directly into the environment while others are released over time from products used in homes and workplaces. Examples of toxic ingredients include volatile organic compounds such as solvents and formaldehyde which are found in an extremely wide range of products, from automotive fuels to household's cleaners and building materials. Avoiding exposure may require significant changes in lifestyle and in the living environment.

A significant proportion of toxins released in the environment find their way into the food chain and water supplies. Numerous studies have shown that contamination of residential water supplies in the U.S. is a serious and widespread problem. Toxicological testing of water samples can be used to identify the presence of certain toxins. The use uncontaminated water sources or of water purification system can be an effective means of reducing xenobiotic exposure.

Foods represent the most common source of exposure to xenobiotics. Approximately 3,000 chemicals are used by the food industry during processing. An additional 12,000 chemicals are used in food packaging materials. Numerous studies have found pesticide residues in a significant percentage of food samples. The use of organically grown and unprocessed foods can be an effective means of reducing exposure to food borne toxins.

Xenobiotics are introduced into the environment and may reach several sites and places all over the world. They can be distributed in water, soil, air and biota. To estimate the exposure of xenobiotics to organisms and to understand which chemical reactions taken place in each compartment, the concentrations of semeiotics in the compartments and the distribution of the xenobiotics over the different compartments are of paramount importance.

Environmental Chemistry does not concern the emissions of xenobiotic chemicals into the environment. It examines the distribution by geochemical and biological processes. Wind speed and direction are important for the (global) distribution in air, water velocity and direction for the (global) distribution in water, and transport phenomena in soil for the (more locally) distribution in soil. Organisms take up xenobiotics from their environment, the route of uptake being dependent on habitat and species. In addition, organisms may disrtribute xenobiotics globally by migration.