UNIT-VI : REPRODUCTION

CHAPTER-1
SEXUAL REPRODUCTION IN FLOWERING PLANTS

TOPIC-1
Sexual Reproduction in Flowering Plants

Revision Notes

- **Flower**
  - Flowers are the site of sexual reproduction in flowering plants.
  - Parts of a typical angiospermic flower are : sepals, petals, stamens and pistils.
  - The four whorls of the flower are attached on a central axis called thalamus.
  - A flower can be bisexual (contains both male and female reproductive parts) or unisexual (only one of the reproductive parts is present).
- **Male Reproductive Structures**

  Androecium (Whorl of Stamens)
  - Androecium consists of a whorl of stamens.
  - The number and length of the stamens are variable in flowers of different species.
  - A stamen has three parts namely, anther, filament and connective.

  (a) Anther
  - It is the terminal and bilobed part of stamens attached with filament. A bilobed anther is called dithecous.
  - Each lobe has two pollen sacs or microsporangia. Therefore, the anther is tetrasporangiate.
  - A longitudinal groove runs lengthwise separating the theca.

  (b) Filament
  - It is the long and slender stalk part of the stamen.
  - Its proximal end is attached to the thalamus or petals of the flower.

  (c) Connective
  - The structure which connects the anther lobes is known as connective.
- **Transverse section of an anther**
  - The anther is tetragonal in a structure consisting of four microsporangia or pollen sacs located at the corners, two in each lobe.
  - The microsporangia develop to become pollen sacs.
  - They extend longitudinally throughout the length of an anther.
  - These are packed with pollen grains.
- **Structure of microsporangium or pollen sac**
  - It is circular and is generally surrounded by wall layers namely,

    (a) Epidermis **(b)** Endothecium

    (c) 2 or 3 Middle layers **(d)** Tapetum
  - The first two layers perform the function of protection and help in dehiscence of anther to release the pollens.
  - The middle layers and the innermost layer, (tapetum) nourishes the developing pollen grains.

- The cells of the tapetum possess dense cytoplasm and more than one nuclei.
- When the anther is young, a group of compactly arranged homogenous cells called sporogenous tissues occupies the centre of each microsporangium.

➢ **Microsporogenesis**

- When the anther develops, each cell of sporogenous tissue undergoes meiotic division to form microspore tetrads.
- Each cell of sporogenous tissue is a microspore mother cell (MMC) or pollen mother cell (PMC).
- The process of formation of microspores from a pollen mother cell (PMC) through meiosis is called microsporogenesis.

➢ **Dehiscence of anther**

- The microspores get arranged in a group of four cells and each group is called microspore tetrad.
- As the anthers mature and dehydrate, the microspores dissociate from each other and develop into pollen grains.
- From each microsporangium, thousands of pollen grains are formed and released due to the dehiscence of anther.

➢ **Pollen grain (Male gametophyte)**

- Pollen grain germinate and give rise to male gametophyte.
- These are spherical, measuring about 25-50 micrometers in diameter.
- Pollen grains are well preserved as fossils due to the presence of sporopollenin, a tough, resistant and stable material.
- A pollen grain has a two-layered wall namely, exine and intine.

(a) Exine

- Exine is the hard outer layer which is made up of sporopollenin.
- The sporopollenin is one of the most resistant organic materials.
- It can withstand high temperature and strong acids and alkali.
- It cannot be degraded by enzymes.
- The exine has apertures called germ pores where sporopollenin is absent.

(b) Intine

- It is the inner, thin and continuous layer that is made up of cellulose and pectin.
- A mature pollen grain contains two cells namely, vegetative cell and generative cell.

(i) Vegetative cell

- It is the bigger cell having abundant food reserve and a large irregularly shaped nucleus.

(ii) Generative cell

- It is the smaller cell that floats in the cytoplasm of the vegetative cell.
- It is spindle shaped with dense cytoplasm and a nucleus.
- The pollen grains are generally shed at the 2-celled stage in flowering plants.
- In other plants, the generative cell divides mitotically to give rise to the two male gametes before pollen grains are shed in a 3-celled stage.
- Once they are shed, pollen grains have to land on the stigma before they lose viability.
- The period of pollen grains remaining viable varies and depends on the prevailing temperature and humidity.
- The viability of pollen grains of some cereals such as rice, wheat, etc. is 30 minutes while some members of Leguminosae, Rosaceae & Solanaceae have viability for months.
- Pollen grains of some plants like *Parthenium* are allergic for some people leading to chronic respiratory disorders such as asthma, bronchitis, etc.
- Pollen grains are rich in nutrients.
- Pollen tablets are used as food supplements.
- Pollen consumption in the form of tablets and syrups increases the performance of athletes and race horses.
- It is possible to store pollen grains for years in liquid nitrogen (–196°C).
- The pollens stored in the pollen banks for crop breeding programmes.

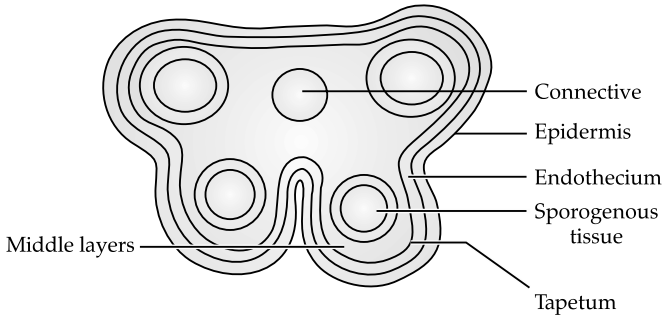


Fig 2.1 : Transverse Section of a young anther

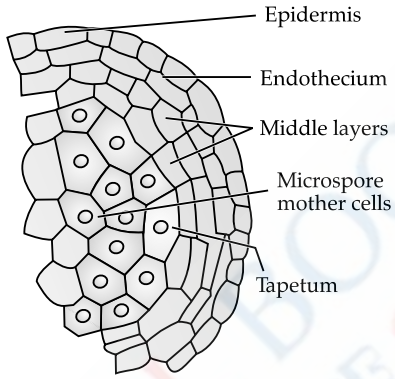


Fig 2.2 : Enlarged view of an microsporangium

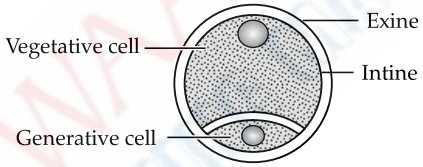


Fig 2.3 : Structure of two-celled male gametophyte (pollen grain)

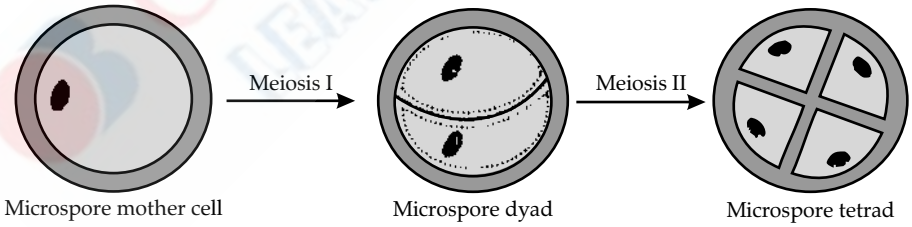


Fig 2.4 : Microsporogenesis

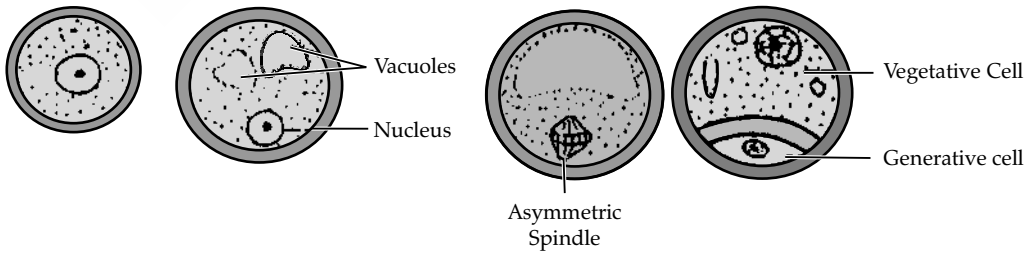


Fig 2.5 : Stages of a microspore maturing into a pollen grain

➢ **Female Reproductive Structures**
Gynoecium (Pistil)
- It represents the female reproductive part of the flower.

- If, it consists of a single pistil or carpel then, it is known as monocarpellary or if, it has more than one pistil or carpel then, it is called multicarpellary.
- When there is more than one carpel, they may be fused then the pistil is known as syncarpous or may be free then, it is known as apocarpous.
- Each carpel has three parts namely stigma, style and ovary.

(a) Stigma

It is a landing platform for pollen grains.

(b) Style

It is an elongated slender part beneath the stigma.

(c) Ovary

- It is the basal swollen part of the carpel.
- Inside the ovary is the ovarian cavity called the locule where the placenta is located.
- Placenta contains the ovules or megasporangia.
- The number of ovules in an ovary may be one as seen in wheat, paddy, mango, etc., or many as seen in papaya, watermelon, orchids, etc.

➢ **Megasporangium (Ovule)**

- It is a small structure attached to the placenta by a stalk called the funicle.
- The junction where the body of the ovule and funicle fuse is called the hilum.
- Each ovule has one or two and some times three protective coverings called integuments.
- Integuments encircle the ovule except at the tip where a small opening called micropyle is organized.
- Opposite to the micropylar end is the chalaza which is the basal part of the ovule.
- Within the integuments, there is a mass of cells called nucellus which contains reserve food materials.
- Inside the nucellus there is an embryo sac, which is also called as the female gametophyte.
- An ovule has a single embryo sac usually formed from a single haploid megaspore.

➢ **Megasporogenesis**

- The formation of haploid megaspores from the diploid megaspore mother cell (MMC) as a results of meiosis is called megasporogenesis.
- A single megaspore mother cell is differentiated in the micropylar region of the nucellus.
- The megaspore mother cell is a large cell containing dense cytoplasm and a prominent nucleus.
- The megaspore mother cell undergoes meiotic division resulting in the production of four haploid megaspores.

➢ **Female gametophyte (Embryo sac)**

- In most of the flowering plants, only one megaspore is functional while the other three degenerate.
- The functional megaspore develops into the female gametophyte or embryo sac.
- This method of embryo sac formation from a single megaspore is termed as monosporic development.

➢ **Development of Female gametophyte**

- The nucleus of the functional megaspore divides mitotically to form two nuclei which move towards the opposite poles, forming a two-nucleated embryo sac.
- Two more sequential mitotic nuclear divisions result in the formation of the four-nucleated and later the eight-nucleated stages of the embryo sac
- These divisions are strictly free nuclear, *i.e.* nuclear divisions are not followed immediately by cell wall formation.
- After eight-nucleate stage, the organization of the typical female gametophyte or embryo sac takes place.
- Generally six of the eight nuclei are surrounded by cell walls and organized into cells.
- The remaining two nuclei called the polar nuclei are found below the egg apparatus in the large central cell.

➢ **Distribution of the cells within the embryo sac**

- The three cells consisting of two synergids and one egg cell which are grouped at the micropylar end constitute the egg apparatus.
- The synergids have special cellular thickenings at the micropylar tip called filiform apparatus.
- The filiform apparatus helps to guide the pollen tubes into the synergid.

- Three cells at the chalazal end organize as the antipodals.
- Thus, a typical mature angiosperm embryo sac at maturity is eight-nucleate and seven-celled.

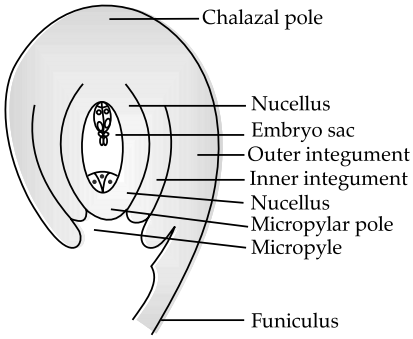


Fig 2.6 : A diagrammatic view of a typical anatropous ovule

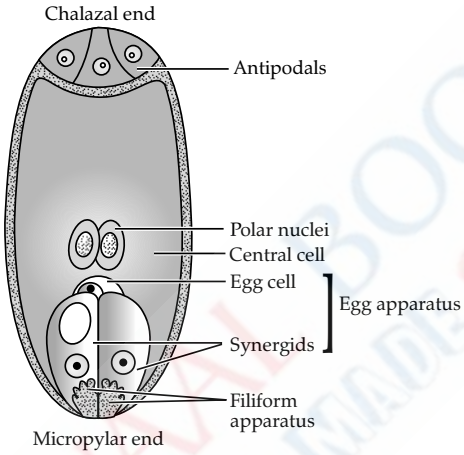


Fig 2.7 : A diagrammatic view of the mature embryo

TOPIC-2
Pollination and Fertilization

Revision Notes

➢ **Modes of Pollination**

- The process of transfer of pollen grains from the anther to the stigma of a pistil is known as pollination.
- There are few external agents which help the plants for pollination to take place.
- The Pollination is of three types based on the source of pollens namely,

(a) Autogamy **(b)** Geitonogamy **(c)** Xenogamy

➢ **Autogamy**

- When the pollen grains are transferred from the anther to the stigma of the same flower, it is known as autogamy.
- In flowers with exposed anthers and stigma, a complete autogamy is rare and hence the anthers and stigma should lie close to each other to enable self-pollination. Along with this there should be synchrony in pollen release and stigma receptivity.
- Plants like *Viola* (common pansy), *Oxalis* and *Commelina* produce two types of flowers namely Chasmogamous flowers and Cleistogamous flowers.

(a) Chasmogamous flowers

- They are similar to flowers of other species with exposed anthers and stigma.

(b) Cleistogamous flowers

- They do not open at all.
- Anthers and stigma lie close to each other.
- They are autogamous as there is no chance of cross-pollination.
- When anthers dehisce in the flower buds, pollen grains come in contact with the stigma for pollination.
- Cleistogamous flowers produce assured seedset even in the absence of pollinators.

➢ **Geitonogamy**

- When the pollen grains are transferred from the anther to the stigma of another flower of the same plant, it is known as geitonogamy.
- It involves pollination with the help of a pollinating agent. It is structurally cross-pollination but genetically self-pollination.
- It is genetically similar to autogamy because the pollen grains come from the same plant.

➢ **Xenogamy**

- When the pollen grains are transferred from anther to the stigma of a different plant, it is known as xenogamy. It brings about genetically different types of pollen grains to the stigma.

➢ **Agents of pollination :**

- There are two types of agents of pollination namely :

(a) Biotic agents **(b)** Abiotic agents

➢ **Abiotic Agents**

- There are two abiotic agents namely, wind and water which help pollination to takes place.

➢ **Pollination by Wind**

- The pollination taking place by the wind is called anemophily.
- Wind and water pollinated flowers are not very colourful and do not produce nectar.
- Wind pollinated flowers often have a single ovule in each ovary.
- Numerous flowers remain packed into an inflorescence.
- Examples – In Corn cob, the tassels are the stigma and style which wave in the wind to trap pollen grains. Wind pollination is commonly seen in grasses.

➢ **Characteristics of Anemophilous flowers**

- The flowers produce an enormous amount of pollen.
- The pollen grains are light and non-sticky so that they can be transported in wind currents.
- They often possess well-exposed stamens for easy dispersal of pollens into wind currents.
- They have large, feathery and sticky stigma to trap air-borne pollen grains.

➢ **Pollination by Water**

- The pollination taking place by water is called hydrophily.
- It is limited to about 30 genera, mostly monocotyledons.
- In *Vallisneria*, the female flowers reach the surface of the water by the long stalk and the male flowers or pollen grains are released on to the surface of the water. These male flowers or pollen grains are carried by water currents and reach the female flowers.
- In seagrasses, the female flowers remain submerged in water and the long, ribbon-like pollen grains are carried inside the water and reach the stigma.
- The pollen grains of most of the water-pollinated species have a mucilaginous covering to protect from wetting.

- Not all aquatic plants use hydrophily. For example, in aquatic plants like water hyacinth, water lily, etc., the flowers emerge above the level of water for entomophily or anemophily *i.e.,* for pollination to takes place by insects or wind.
- It is seen in *Vallisneria* & *Hydrilla* (freshwater), *Zostera* (marine sea-grasses), etc.

➢ **Biotic Agents**

- Some flowering plants use animals as pollinating agents like Bees, butterflies, flies, beetles, wasps, ants, moths, birds (sunbirds and hummingbirds) bats, some primates (lemurs), arboreal (tree-dwelling) rodents, reptiles (gecko lizard & garden lizard) etc.
- When the pollination takes place by insects, it is known as entomophily.
- Often flowers of animal pollinated plants are specifically adapted for a particular species of animal.
- When the animal comes in contact with the anthers body gets a coating of pollen grains and when it comes in contact with the stigma, it results in pollination.
- Some plants provide safe places as a floral reward to lay eggs as seen in *Amorphophallus*, the tallest flower.
- There is a very close obligatory symbiotic relationship between the species of moth (*Pronuba*) and the plant *Yucca*. They cannot complete their life cycles without each other. The moth deposits its eggs in the locule of the ovary and the flower gets pollinated by the moth. The larvae of the moth come out of the eggs as the seeds start developing.
- Many insects consume pollen or nectar without bringing about pollination. They are called pollen/nectar robbers.

➢ **Characteristics of Entomophilous Flowers**

- Flowers are large, colourful, fragrant and rich in nectar.
- When the flowers are small, they form inflorescence to make them visible.
- The flowers pollinated by flies and beetles secrete foul odours to attract these animals.
- The pollen grains are generally sticky.

➢ **Outbreeding Devices (Devices for promoting Cross-Pollination)**

- To avoid self-pollination, cross-pollination is encouraged in plants as follows :

(a) Avoiding Synchronization

- In some species, pollen release and stigma receptivity are not synchronized.
- Either the pollen is released before the stigma becomes receptive or the stigma becomes receptive before the release of pollen *i.e.*, the anther and stigma mature at different times. This phenomenon is called dichogamy. It prevents autogamy.

(b) Arrangement of Anther and Stigma at different Positions

- In some species, the arrangement of anther and stigma at different positions prevents autogamy.

(c) Self-incompatibility

- It is a genetic mechanism that prevents pollen of one flower to germinate on the stigma of the same flower on of the same plant due to the presence of similar sterile genes in pollen and stigma.

(d) Production of Unisexual Flowers (Dicliny)

- Monoecious plants such as castor and maize, where the male and the female flowers are present on the same plant prevents autogamy but not geitonogamy. On the other hand, dioecious plants like papaya, where the male and female flowers are present on different plants prevents both autogamy and geitonogamy.

➢ **Pollen-pistil Interaction**

- It is a dynamic process involving pollen recognition followed by promotion or inhibition of the pollen.
- This interaction takes place through the chemical components produced by them.
- If the pollen is compatible, then the pistil accepts it and promotes post-pollination events.

- The pollen grain germinates on the stigma to produce a pollen tube through one of the germ pores.
- The contents of the pollen grain move into the pollen tube.
- The pollen tube grows through the tissues of the stigma and style and reaches the ovary.
- If the pollen is incompatible, then the pistil rejects the pollen by preventing pollen germination on the stigma or the pollen tube growth in the style.
- In some plants, the pollen grains are shed at the two-celled stage, the generative cell divides and forms the two male gametes during the growth of the pollen tube on the stigma.
- In plants that shed pollen in the three-celled stage, the pollen tubes carry two male gametes from the beginning.
- The pollen tube, after reaching the ovary, enters the ovule through the micropyle chalaza/integuments and then enters one of the synergids through the filiform apparatus.
- The filiform apparatus present at the micropylar part of the synergids guides the entry of the pollen tube.
- A plant breeder can manipulate pollen-pistil interaction, even in incompatible pollinations, to get desired hybrids.

➢ **Artificial Hybridization**

- It is one of the major approaches of crop improvement programme by using desired pollen grains for pollination.
- This is achieved by emasculation and bagging techniques.
- Emasculation is the removal of anthers by using forceps from the bisexual flower bud of female parent before the anther dehiscense.
- The emasculated flowers are then covered with a suitable bag made up of butter paper to prevent contamination of its stigma with unwanted pollen. This is called bagging.
- When the stigma attains receptivity, the mature pollen grains collected from anthers of the male parent are dusted on the stigma. Then the flowers are rebagged and allowed to develop the fruits.
- If the female parent produces unisexual flowers, there is no need for emasculation.
- The female flower buds are bagged before the flowers open.
- When the stigma becomes receptive, pollination is carried out using the desired pollen and the flower rebagged.

➢ **Double Fertilization**

- The pollen tube after entering one of the synergids releases its contents including the two male gametes into the cytoplasm of the synergid.
- One of the male gametes moves towards the egg cell and fuses with its nucleus by the process of syngamy to form a diploid cell called the zygote.
- The other male gamete moves towards the two polar nuclei located in the central cell and fuses with them to produce a triploid primary endosperm nucleus (PEN).
- As this involves the fusion of three haploid nuclei, it is called triple fusion.
- Since two types of fusions viz. syngamy and triple fusion take place in an embryo sac, it is called double fertilization.
- The central cell after triple fusion becomes the primary endosperm cell (PEC) and develops into the endosperm while the zygote develops into an embryo.
- It is an event unique to flowering plants.

TOPIC-3
Post-Fertilization Changes and Special Modes of Reproduction

Revision Notes

Embryo and its Development

➢ **Post-fertilization Events**

- The development of endosperm and embryo, the maturation of ovule(s) into seed(s) and ovary into fruit are post-fertilization events.

➢ **Endosperm Development**

- The primary endosperm cell divides repeatedly by mitosis to form a triploid endosperm tissue.
- Endosperm cells are filled with reserve food materials that are used for the nutrition of the developing embryo.
- During the endosperm development, the primary endosperm nucleus undergoes successive mitotic nuclear divisions to give rise to free nuclei. This stage is called free-nuclear endosperm.
- Then the endosperm becomes cellular due to the cell wall formation.
- For example, the tender coconut water is a free-nuclear endosperm that is made up of thousands of nuclei and the surrounding white kernel is the cellular endosperm.

➢ **Embryo Development**

- The embryo develops at the micropylar end of the embryo sac where the zygote is situated.
- The zygotes divides only after the formation of a certain amount of endosperm to provide nutrition to the developing embryo.
- The development of embryo is similar in monocotyledons and dicotyledons up to the octant stage.
- The zygote gives rise to the proembryo and subsequently to the globular, heart-shaped and mature embryo.

➢ **Dicotyledonous Embryo**

- It has a central embryonal axis and two lateral cotyledons.
- The portion of the embryonal axis above the level of cotyledons is the epicotyl, which terminates into the plumule (stem tip).
- The cylindrical portion below the level of cotyledon is hypocotyl that terminates into the radicle (root tip).
- The root tip is covered with a root cap.

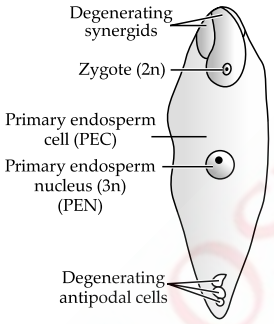


Fig 2.8 : Fertilized embryo sac showing zygote and primary endosperm nucleus

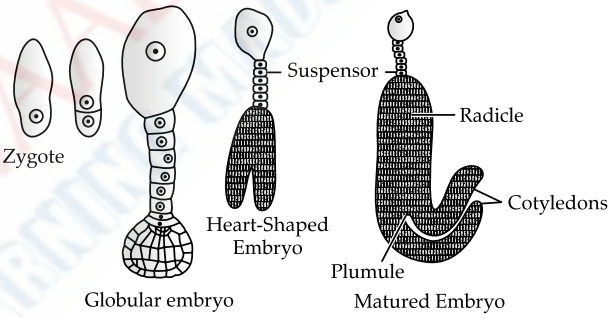


Fig. 2.9 : Stages in embryo development in a dicot

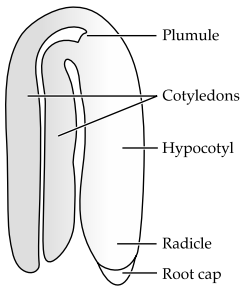


Fig 2.10 : A typical dicot embryo

➢ **Monocotyledonous Embryo**

- They possess only one cotyledon.
- In the grass family, the cotyledon is called the scutellum which is situated lateral to the embryonal axis.
- At its lower end, the embryonal axis has the radicle and root cap enclosed in an undifferentiated sheath called coleorhiza.

- The portion of the embryonal axis above the level of attachment of the scutellum is the epicotyl.
- It has a shoot apex and a few leaf primordia enclosed in a hollow foliar structure called coleoptile.

➢ **Seed**

- Seed is the final product of sexual reproduction.
- It is the fertilized ovule formed inside fruits.
- It consists of the seed coat(s), cotyledon(s) and an embryonal axis.
- The cotyledons are simple, thick and swollen due to the storage of food as seen in most of the dicots.
- Mature seeds may be non-albuminous or albuminous.

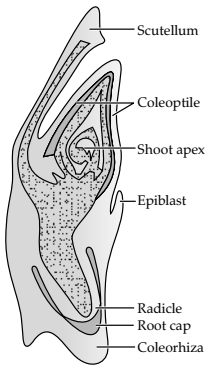
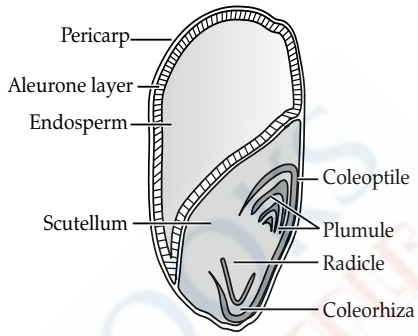


Fig 2.11 : L.S of an embryo of grass



L.S. of Maize grain

Fig 2.12 : L.S grain of maize

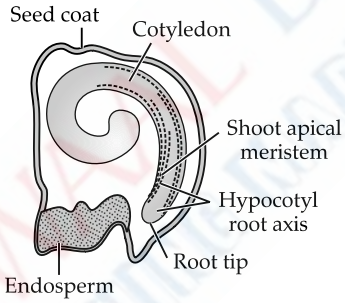


Fig 2.13 : L.S. monocot seed of onion

➢ **Non-albuminous or Non-endospermic Seeds**

- These seeds have no residual endosperm as it is completely consumed during embryo development.

Examples - pea, groundnut, beans.

➢ **Albuminous or Endospermic Seeds**

- These seeds retain a part of the endosperm as it is not completely used up during embryo development.

Examples, wheat, maize, barley, castor, coconut, sunflower.

- In some seeds like black pepper, beet, etc., the remnants of nucellus also persistent. It is called the perisperm.
- Integuments of ovules harden as tough protective seed coats.
- It has a small pore (micropyle) through which oxygen and water enter into the seed during germination.
- As the seed matures, its water content gets reduced and the seeds become dry (10-15 % moisture by mass). The general metabolic activity of the embryo slows down.
- The embryo may enter a state of inactivity (dormancy).
- If favourable conditions are available such as adequate moisture, oxygen and suitable temperature, they germinate.

➢ **Fruit**

- The ovary develops into a fruit after pollination and fertilization.
- The transformation of ovules into seeds and ovary into fruit proceeds simultaneously.
- The wall of the ovary develops into a pericarp.
- The fruits may be fleshy as seen in guava, orange, mango, etc., or may be dry as seen in groundnut, mustard, etc.,
- Many fruits have mechanisms for the dispersal of seeds.

- Fruits are of two types namely :
  - **(a) True fruits :** When the fruit develops only from the ovary and other floral parts degenerate and fall off, they are called true fruits. Examples- mango, maize, grape.
  - **(b) False fruits :** When parts of a flower other than the ovary also contribute to the fruit formation, they are called false fruits. Examples– apple, strawberry, cashew, etc.
- In some species such as banana, the fruits develop without fertilisation, these fruits are called parthenocarpic fruits.
- Parthenocarpy can be induced through the application of growth hormones. Such fruits are seedless.

➢ **Advantages of Seeds**
- The pollination and fertilization processes are independent of water while the seed formation is more dependable.
- Seeds have better adaptive strategies for dispersal to new habitats and help the species to colonize in other areas.
- They have food reserves and so young seedlings are nourished until they are capable of photosynthesis.
- The hard seed coat protects the young embryo.
- Since seeds are the products of sexual reproduction, they generate new genetic combinations leading to variations.
- The dehydration and dormancy of mature seeds are crucial for the storage of seeds.
- It can be used as food throughout the year and also to raise a crop in the next season.

➢ **Viability of Seeds after Dispersal**
- In a few species, the seeds lose viability within a few months or live for several years.
- Some seeds remain alive for hundreds of years.
- The oldest is lupine (*Lupinus arcticus*) excavated from Arctic Tundra. The seed germinated and flowered after an estimated record of 10,000 years of dormancy.
- 2000 years old viable seed is of the date palm (*Phoenix dactylifera*) discovered during the archeological excavation at King Herod's palace near the Dead Sea.

➢ **Apomixis and Polyembryony**
- Apomixis is (apo = without; mixis = mixing together) means the production of seeds without fertilization.
- It is seen in some species of Asteraceae and grasses.
- The apomixis is a form of asexual reproduction that mimics sexual reproduction.
- The occurrence of more than one embryos in a seed is called polyembryony.

➢ **Development of Apomictic Seeds**
- In some species, the diploid egg cell is formed without reduction division and develops into the embryo without fertilization.
- In species like Citrus and Mango varieties, some of the nucellar cells surrounding the embryo sac divide and protrude into the embryo sac and develop into the embryos. Hence, in these species, each ovule contains many embryos.

➢ **Importance of Apomixis in Hybrid Seed Industry**
- Hybrid seeds have to be produced every year.
- If the seeds collected from hybrids are sown, the plants in the progeny will segregate and lose hybrid characters.
- The production of hybrid seeds is costly. Hence the cost of hybrid seeds is also expensive for the farmers.
- If the hybrids are made into apomict, there is no segregation of characters in the hybrid progeny. This helps farmers to use the hybrid seeds to raise new crop year after year without losing hybrid characteristics.

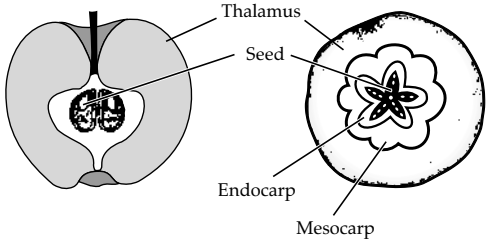


Fig 2.14 : Sectional view of an apple

Know the Terms

- **Anemophily :** Pollination by wind is called anemophily.
- **Apomixis :** It is production of seeds without involving the process of meiosis and syngamy.
- **Chiropterophily :** Pollination by bat is called chiropterophily.
- **Coleorhiza :** It is undifferentiated sheath that encloses the radicle and root cap in a monocot seed.
- **Emasculation :** It is the process of removal of anthers (using forceps) from the bisexual flower bud without affecting the female reproductive part *i.e.,* pistil.
- **Entomophily :** Pollination by insects is called entomophily.
- **False fruits :** Fruits that develop from accessory parts other than ovary are called as false fruits.
- **Geitonogamy :** It is the transfer of pollen grains from the anther to the stigma of another flower of the same plant.
- **Hydrophily :** Pollination by water is called hydrophily.
- **Megasporogenesis :** It is the process of formation of the four megaspores from the megaspore mother cell (MMC) in the region of nucellus through meiosis.
- **Microsporogenesis :** It is process of formation of microspore from a pollen mother cell (PMC) through meiosis.
- **Ornithophily :** Pollination by bird is called ornithophily.
- **Parthenocarpic fruits :** Fruits that develop without fertilization are called parthenocarpic fruits.
- **Perisperm :** Remnants of nucellus in matured seed are known as perisperm.
- **Pollen-pistil interaction :** All the events – from pollen deposition on the stigma until pollen tubes enter the ovule – are together referred as pollen–pistil interaction.
- **True fruits :** Fruits that develop from the ovary are called true fruits.
- **Pollination :** It is the process of transfer of pollen grains from the anther to the stigma of a pistil.
- **Cross pollination :** It is the process of transfer of pollen grain from one flower to the stigma of another flower.
- **Self-Pollination or autogamy :** It is the transfer of pollen from anther to the stigma of the same flower.
- **Xenogamy :** It is the transfer of pollen grains from anther to the stigma of a different plant.
- **Bisexual flower :** Flower which contain both male (stamens) and female (carpels or pistils) reproductive parts in it.
- **Unisexual flower :** Flower which contain only one i.e., either male or female reproductive parts in it.
- **Out breeding devices :** Devices that have been developed by the flowering plants to avoid self-pollination and to encourage cross-pollination.
- **Polyembryony :** It is the presence of more than one embryo in a seed.
- **Testa :** It is the outer layer of the seed coat that develops into the integument of the ovule.
- **Tegmen :** It is the inner layer of the seed coat that develops from the integument of the ovule.
- **Perisperm :** Remnants of nucellus in matured seed are known as perisperm.

❑❑

CHAPTER-2
HUMAN REPRODUCTION

TOPIC-1
Human Reproduction System

Revision Notes

Reproductive System

- **Male Reproductive System**
- **It consists of :**
  (a) A pair of testes
  (b) Accessory ducts
  (c) Accessory glands
  (d) External genitalia

- **Testes**
  - Testes are the primary sex organs that produce sperms and testosterone hormone.
  - Testes are located in the scrotum present in between upper thighs.
  - The low temperature (2 – 2.5°C less than the normal internal body temperature) in the scrotum helps for the proper functioning of testes and spermatogenesis.
  - Each testis is oval in shape and has about 250 (200 – 300) compartments called testicular lobules.
  - Each lobule is filled with connective tissue and contains 1-3 coiled yellow seminiferous tubules in which sperm are produced.
  - Seminiferous tubule is lined internally with spermatogenic cells called spermatogonia or primary male germ cells and sertoli cells or supporting cells.
  - Spermatogonia undergo meiotic divisions and leads to sperm formation.
  - Sertoli cells give shape and nourishment to developing spermatogenic cells and therefore also called as **nurse cells.**
  - The regions outside the seminiferous tubules are the interstitial spaces which contain small blood vessels and interstitial cells or Leydig cells.
  - The Leydig cells are endocrine in nature and secrete testicular hormones called androgens.
  - Immunologically competent cells are also present.
- **Accessory Ducts**
  - The duct system includes rete testis, vasa efferentia, epididymis and vas deferens.
  - The seminiferous tubules open into the vasa efferentia through rete testis.
  - The vasa efferentia open into the epididymis.
  - The epididymis leads to vas deferens that ascends into the abdomen and loops over the urinary bladder.
  - It receives a duct from the seminal vesicle and opens into the urethra as the ejaculatory duct.
  - These ducts store and transport the sperms from the testis to the outside through urethra.
  - The urethra originates from the urinary bladder and extends through the penis to its external opening called the urethral meatus.
- **Accessory Male Genital Glands**
  - It includes paired seminal vesicles, prostate and paired bulbourethral glands (Cowper's glands).
  - The secretions of these glands constitute the seminal plasma, which is rich in fructose, calcium and certain enzymes.
  - Seminal vesicles produce seminal fluid and form 60 – 70% of semen.
  - The secretion of bulbourethral glands is alkaline and rich in mucus. It helps in the lubrication of the penis, supplies nutrient to sperms and provides an alkaline medium to counteract the acidity of the uterus.
- **External Genitalia**
  - The penis is the male external genitalia.
  - It is made up of special tissue that helps in the erection of the penis to facilitate insemination.
  - The enlarged end of the penis is called the glans. The penis is covered by a loose fold of skin called foreskin.

IMPORTANT DIAGRAMS

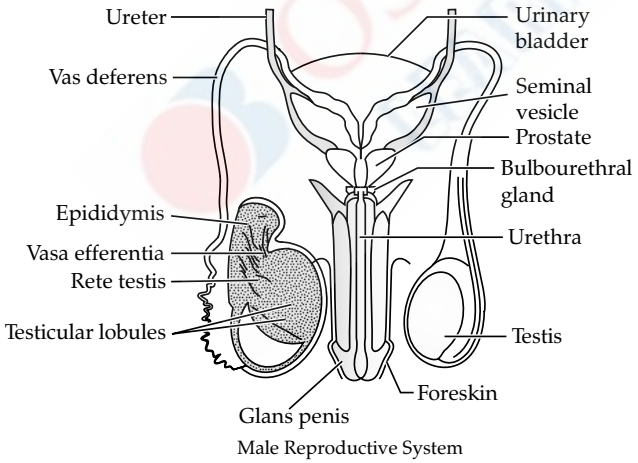


Fig 3.1 : Human Male Reproductive System

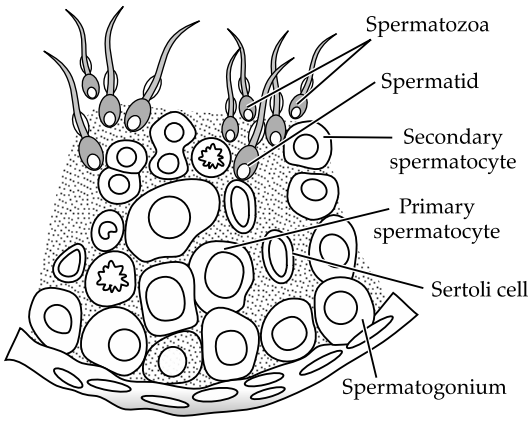


Fig 3.2 : Sectional view of human seminiferous tubule

- **The Female Reproductive System**
  - It includes a pair of ovaries, accessory ducts and external genitalia.
- **Ovaries**
  - They are the primary female sex organs that produce ova or the female gametes. It secretes many steroid ovarian hormones such as estrogen and progesterone.
  - Ovaries are located on both sides of the lower abdomen.
  - Each ovary is about 2-4 cm in length.
  - The ovaries are connected to the pelvic wall and uterus by ligaments.
  - Each ovary is covered by a thin epithelium which encloses the ovarian stroma.

- The stroma has outer cortex and an inner medulla.
- The ovary contains groups of cells known as Ovarian or Graafian follicles.
- Each follicle carries a centrally placed ovum.

➢ **Accessory Ducts**
- It includes two oviducts or fallopian tubes, cervix, a uterus and vagina.
- Each oviduct is 10-12 cm long and has four parts namely, infundibulum, ampulla, isthmus and uterine part.

(a) Infundibulum
- It is the funnel-shaped opening provided with many finger-like fimbriae for catching released ovum.
- It helps to collect the ovum after its release from the ovary.

(b) Ampulla
- The infundibulum leads to the curved and dilated part called the ampulla.

(c) Isthmus
- It is the last straight part of the oviduct.
- It has a narrow lumen and joins the uterus.

(d) Uterine part
- It is about 1 cm long part of the oviduct which passes into the uterus.

➢ **Uterus**
- It is single and also called the womb.
- The shape of the uterus is like an inverted pear.
- It is supported by ligaments attached to the pelvic wall.
- The uterus opens into the vagina through a narrow cervix.
- The cavity of the cervix is called the cervical canal which along with the vagina forms the birth canal.
- The wall of the uterus is thick and muscular and is differentiated into three layers of tissue namely,

  (a) The external thin membranous perimetrium.

  (b) The middle thick layer of smooth muscle, myometrium.

  (c) The inner glandular layer called the endometrium.
- The endometrium undergoes cyclic changes during the menstrual cycle while the myometrium exhibits strong contraction during delivery of the baby.
- The vagina opens to the exterior between the urethra and anus.
- The lumen of the vagina is lined by a glycogen-rich mucous membrane consisting of sensitive papillae and Bartholin's glands.
- The secretions of Bartholin's glands lubricate the penis during sexual activity.

➢ **External Genitalia**
- It includes the mons pubis, labia majora, labia minora, hymen and clitoris. The external genitalia are collectively called the vulva.
- Mons pubis is a cushion of fatty tissue covered by skin and pubic hair.
- The labia majora are a pair of large thicker fleshy folds of tissue, which surround the vaginal opening.
- The labia minora are a pair of narrow fleshy folds of tissue found below labia majora.
- The opening of the vagina is often covered partially by a membrane called the hymen.
- The hymen is often torn during the first coitus (intercourse) or accidentally.
- The clitoris is a tiny finger-like structure that lies at the upper junction of the two labia minora above the urethral opening.

➢ **Mammary Glands**
- A pair of mammary glands containing glandular tissue and fat is present in the chest region.
- The glandular tissue of each breast has 15-20 mammary lobes containing clusters of cells called alveoli.
- The cells of alveoli secrete milk which is stored in the cavities or lumen of alveoli.
- The alveoli open into mammary tubules.
- The tubules of each lobe join to form a mammary duct.
- Several mammary ducts join to form a wider mammary ampulla which is connected to the lactiferous duct through which milk is sucked out.

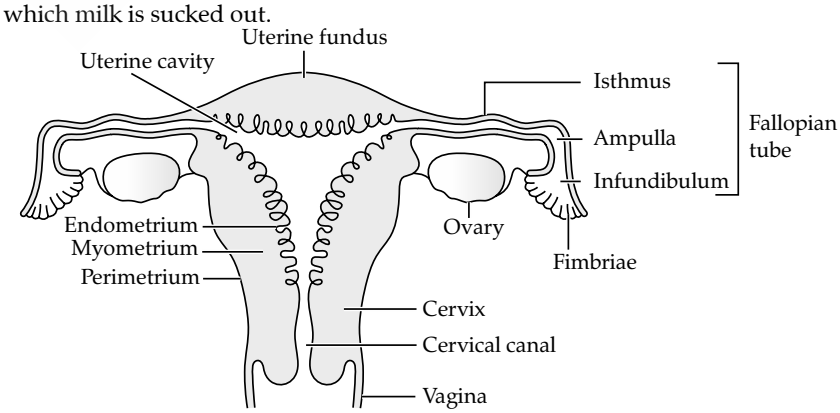


Fig 3.3: Human Female Reproductive system

TOPIC-2
Gametogenesis and Menstrual Cycle

Revision Notes

- **Gametogenesis**
  - The process of formation of gametes or sex cells is known as gametogenesis.
  - It includes spermatogenesis and oogenesis.
- **Spermatogenesis**
  - It is the process of the formation of sperms in seminiferous tubules of testes.
  - It has two stages namely,

    (a) Formation of spermatids

    (b) Spermiogenesis
  - During the formation of spermatids, spermatogonia *i.e.*, sperm mother cells or immature male germ cells produce spermatids.
  - In spermiogenesis, the spermatids are transformed into sperm.
  - Each primary spermatocyte undergoes meiosis-I and produces two haploid secondary spermatocytes.
  - Each secondary spermatocyte divides by meiosis-II and produces two haploid spermatids.
  - Thus, four spermatids are formed from each primary spermatocyte.
  - The spermatids, under the influence of FSH of the anterior pituitary, are converted into spermatozoa. The process is called spermiogenesis.
  - After spermiogenesis, the sperm head become embedded in the Sertoli cells and are finally released from seminiferous tubules. The process of release of mature spermatozoa from the Sertoli cells into the lumen of seminiferous tubules is known as spermiation.
- **Hormones in Spermatogenesis**
  - The hypothalamus releases a large amount of Gonadotropin-releasing hormone (GnRH).
  - GnRH stimulates the anterior pituitary gland to secrete two gonadotropins namely Luteinizing hormone (LH) and Follicle stimulating hormone (FSH).
  - LH acts on the Leydig cells and stimulates the synthesis and secretion of androgens which in turn stimulate the spermatogenesis.
  - FSH acts on the Sertoli cells and stimulates the secretion of some spermatogenic factors which help in the process of spermiogenesis.
- **Structure of Sperm**
  - It is a microscopic structure.
  - A mature sperm measures about 60 μm (0.06 mm) long.
  - A plasma membrane envelops the whole body of sperm.
  - Sperm consists of four parts namely, head, neck, a middle piece and a tail region.

  (a) Head
  - It is oval-shaped, consisting of a nucleus and acrosome.
  - The acrosome is formed from Golgi complex which contains lytic enzymes, that help in fertilization of the ovum.

  (b) Neck
  - Behind the head is a neck containing proximal and distal centrioles.
  - The distal centriole of the neck is connected to the axial filament.

  (c) Middle Piece
  - It is composed of axial filament surrounded by numerous mitochondria and cytoplasm.
  - Mitochondria produce energy for the sperm motility.

  (d) Tail
  - It consists of a central axial filament.
  - The sperm moves in fluid medium and female genital tract by the undulating movement of the tail.
  - Sperms are transported through the accessory ducts.
  - The secretions of the epididymis, vas deferens, seminal vesicle and prostate are essential for maturation and motility of sperms.
  - The seminal plasma and sperms together constitute the semen.
  - The human male ejaculates about 200-300 million sperms during a coitus ejaculation.
  - For normal fertility at least 60% of sperms must have a normal shape and size and 40% of them, must show vigorous motility.

➢ **Oogenesis**

- It is the process of formation and maturation of the ovum.
- It takes place in Graafian follicles.
- It is initiated in embryonic stage when millions of egg mother cells (oogonia) are formed within each ovary.
- No oogonia are formed and added after birth.
- Oogonia multiply to form primary oocytes which enter into prophase-I of the meiosis and get temporarily arrested at that stage.
- Each primary oocyte gets surrounded by a layer of granulosa cells to form a primary follicle.
- A large number of primary follicles degenerate during the phase from birth to puberty.
- Therefore at puberty, only 60,000-80,000 primary follicles are left in each ovary.
- The primary follicles get surrounded by more layers of granulosa cells and a new theca to form secondary follicles.
- The secondary follicles get transformed into a tertiary follicle.
- It has a fluid-filled cavity (antrum).
- The theca layer forms an inner theca interna and an outer theca externa.
- The primary oocyte within the tertiary follicle grows in size and undergoes first unequal meiotic division to form a large haploid secondary oocyte and a tiny first polar body.
- The secondary oocyte retains the nutrient-rich cytoplasm of the primary oocyte.
- It is unknown, whether the first polar body divides further or degenerates.
- The tertiary follicle further changes into the mature follicle (Graafian follicle).
- The secondary oocyte forms a new membrane (zona pellucida).
- The Graafian follicle now ruptures to release the secondary oocyte (ovum) from the ovary. This is called ovulation.

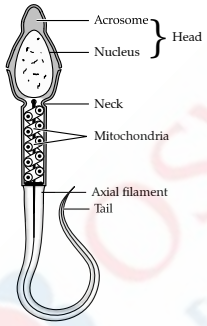


Fig 3.4 : Structure of a Sperm

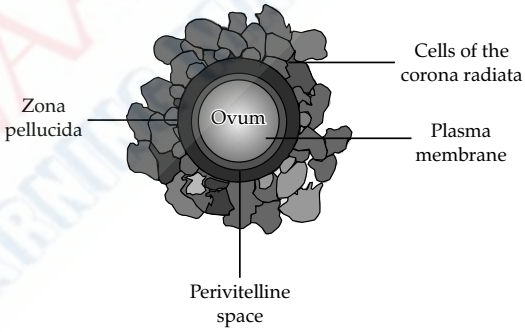


Fig 3.5 : Structure of human ova

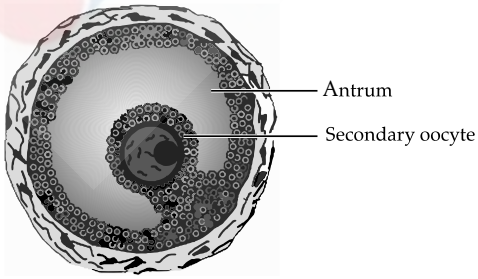


Fig 3.6 : Graafian follicle

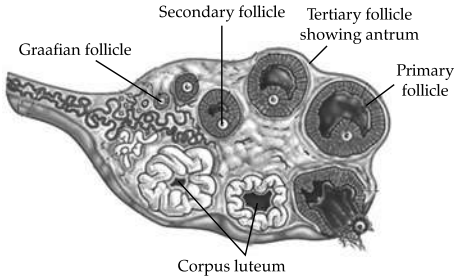


Fig 3.7 : Sectional view of ovary

➢ **Structure of Ovum**

- It is a spherical or oval and non-motile female gamete.
- It is about 0.2 mm in diameter.
- The human ovum is non cleidoic (without shell) and alecithal (without yolk).
- Ovum has four membranes namely,

(a) Plasma membrane (Oolemma) : Innermost layer.

(b) Vitelline membrane : Attached to the plasma membrane.

(c) Zona pellucida : Transparent non-cellular, thick, glycoprotein rich layer found outer to the vitelline membrane.

(d) Corona radiata : Outer layer formed of follicle cells. These cells are held together by a mucopolysaccharide called hyaluronic acid.

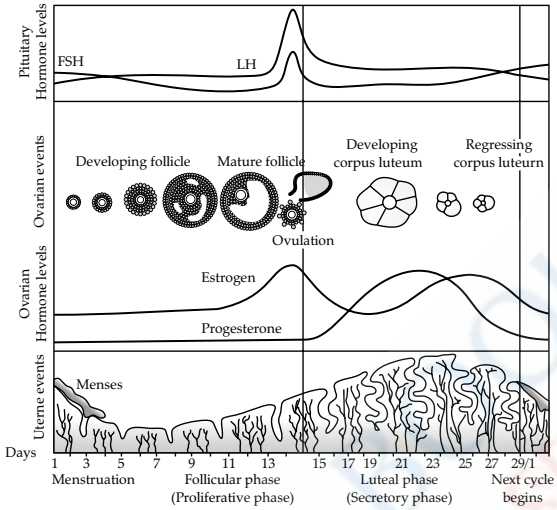


Fig 3.8 : Various events during Menstrual Cycle

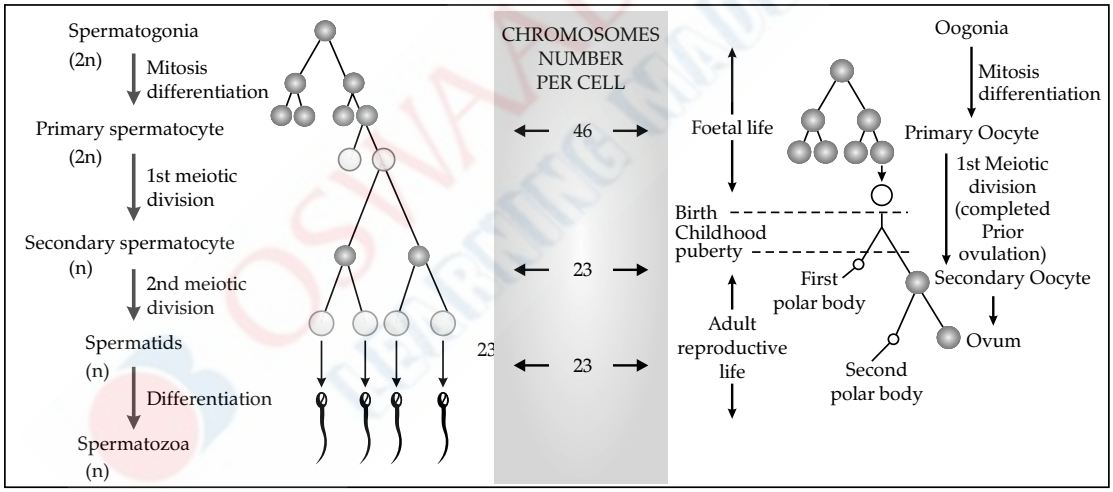


Fig 3.9 : Schematic representation of (a) Spermatogenesis (b) Oogenesis

➢ **Menstrual Cycle**

- The reproductive cycle in the human female and related primates is called the menstrual cycle.
- The first menstruation begins at puberty (at the age of 10-12 years) and is called menarche.
- In human females, menstruation is repeated at an average interval of about 28/29 days and the cycle of events starting from one menstruation till the next one is called the menstrual cycle.
- One ovum is released during the middle of each menstrual cycle.
- The cycle starts with the menstrual phase, when menstrual flow occurs, it lasts for 3-5 days.
- The menstrual flow results due to the breakdown of the endometrial lining of the uterus and its blood vessels which form the liquid that comes out through the vagina.
- Menstruation occurs only if the released ovum is not fertilized.
- Lack of menstruation may be indicative of pregnancy or may also be caused due to some other underlying causes like stress, poor health, etc.
- The menstrual phase is followed by the follicular phase.
- During the follicular phase, the primary follicles in the ovary grow to become a fully mature Graafian follicle and simultaneously, the endometrium of uterus regenerates through proliferation. These changes in the ovary and the uterus are induced by changes in the levels of pituitary and ovarian hormones.

- The secretion of gonadotropins (LH and FSH) increases gradually during the follicular phase and stimulates follicular development as well as secretion of estrogens by the growing follicles.
- Both LH and FSH attain a peak level in the middle of the cycle (about the 14th day).
- Rapid secretion of LH leading to its maximum level during the mid-cycle called LH surge induces rupture of Graafian follicle and thereby the release of an ovum (ovulation).
- The ovulation (ovulatory phase) is followed by the luteal phase during which the remaining parts of the Graafian follicle transform as the corpus luteum.
- The corpus luteum secretes large amounts of progesterone which is essential for the maintenance of the endometrium.
- During pregnancy, all events of the menstrual cycle stop and there is no menstruation.
- In the absence of fertilization, the corpus luteum degenerates. This causes disintegration of the endometrium leading to menstruation, marking a new cycle.
- In human beings, the menstrual cycle ceases at around 50 years of age and is termed as menopause.
- Cyclic menstruation is an indicator of the normal reproductive phase and extends between menarche and menopause.

TOPIC-3
Fertilization and Post-Fertilization Events

Revision Notes

➢ **Fertilization**

- The process of fusion of male gamete (sperm) with the female gamete (ovum) is called fertilization.
- During copulation, semen is released through the penis into the vagina (insemination).
- After insemination, the sperms swim through the cervix and enter into the uterus and reach the ampullary-isthmic junction of the oviduct where fertilization takes place.
- The process of fertilization takes place as follows :

Sperms → vagina → cervical canal → uterus → isthmus

↓

Fertilization ← Ampullary-isthmic Junction

↑

Ovum (from ovary) → fimbriae → infundibulum → ampulla

- Fertilization (sperm + ovum → zygote) occurs only if ovum and sperms are transported simultaneously. So all copulations do not lead to fertilization and pregnancy.
- As soon as sperm contacts with zona pellucida, it induces changes in the membrane that block entry of additional sperms.
- With the help of enzymes of the acrosome, which dissolve the zona pellucida and plasma membrane of the ovum, the sperm enters into the cytoplasm of the ovum. This induces second meiotic division of the secondary oocyte to form a second polar body and a haploid ovum (ootid).
- The haploid nuclei of the sperm and ovum fuse together to form a diploid zygote.

➢ **Implantation**

- The mitotic division (cleavage) starts as the zygote moves through the isthmus of the oviduct towards the uterus and forms 2, 4, 8, 16 daughter cells called blastomeres.
- The embryo with 8-16 blastomeres is called a morula.
- Morula continues to divide and transforms into a large mass of cells called the blastocyst, which moves further towards the uterus.
- The blastomeres in the blastocyst are arranged into an outer layer (trophoblast) and an inner group of cells (inner cell mass) attached to the trophoblast.
- The trophoblast layer then gets attached to the endometrium and the inner cell mass gets differentiated into three germ layers namely, outer ectoderm, middle mesoderm and inner endoderm forming 3-layered structure (gastrula) leading to the formation of the embryo.
- After attachment, uterine cells divide rapidly and cover the blastocyst.

- As a result, the blastocyst becomes embedded in the endometrium of the uterus. This is called implantation.

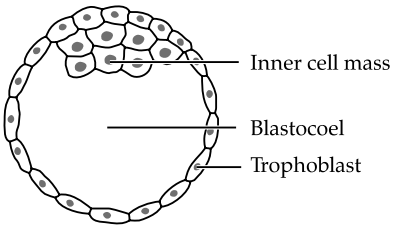


Fig 3.11: Diagram of a Blastocyst

- **Pregnancy and Embryonic Development**
  - After implantation, the finger-like projections called chorionic villi appear on the trophoblast which is surrounded by the uterine tissue and maternal blood.
  - The chorionic villi and uterine tissue become interdigitated with each other and form a structural and functional unit between the developing embryo and the maternal body called the placenta.
  - The placenta is a structural and functional unit between the embryo (foetus) and the maternal body.
  - The placenta is connected to the embryo by an umbilical cord.
  - The umbilical cord helps to transport substances to and from the embryo.
- **Functions of Placenta**
  - It acts as a barrier between the foetus and mother.
  - Soluble inorganic and organic materials, nutrients, hormones, antibodies, etc. can pass through the placenta from the mother to the foetus.
  - It helps in the gas exchange between mother and foetus.
  - It helps to eliminate nitrogenous wastes of foetus.
  - It acts as an endocrine gland by secreting several hormones like human Chorionic Gonadotropin (hCG), human Placental Lactogen (hPL), oestrogens, progesterone and relaxin.
- **Pregnancy**
  - During pregnancy, levels of estrogen, progestogen, cortisol, prolactin, thyroxine, etc. are also increased in maternal blood.
  - They support the foetal growth, metabolic changes in the mother and maintain pregnancy.
  - Three germ layers (ectoderm, endoderm, mesoderm) give rise to all tissues (organs) in adults.
  - The stem cells in inner cell mass have the potency to give rise to all the tissues and organs.
  - Human pregnancy (gestation period) lasts 9 months (for cats : 2 months, dogs : 2 months, elephants : 21 months).
- **Changes in Embryo during Pregnancy**
  - After one month of pregnancy : The heart is formed.
  - End of second month : Limbs and digits are developed.
  - End of 12 weeks (first trimester) : The major organs such as limbs, external genital organs etc., are well developed.
  - During 5^{th} month : The first movement of foetus and appearance of hair on the head.
  - End of 24 weeks (second trimester) : Body is covered with fine hair, eyelids separate and eye lashes are formed.
  - End of 9 months : Ready for delivery.
- **Parturition (Labour) and Lactation**
  - The process of giving birth to young ones after the gestation period of nine months is known as parturition.
  - Parturition is induced by a neuroendocrine mechanism.
  - The signals originating from the foetus and placenta induce mild uterine contractions (foetal ejection reflex). This causes the release of oxytocin from the maternal pituitary.
  - Oxytocin causes stronger uterine muscle contractions which in turn stimulate further secretion of oxytocin. This process is continued leading to the expulsion of the baby out of the uterus through the birth canal.
  - After parturition, the umbilical cord is cut off.
  - The placenta and remnants of the umbilical cord are expelled from the maternal body after parturition. This is called "after birth".
- **Lactation**
  - The mammary glands produce milk towards the end of pregnancy by the process called lactation.
  - The yellowish milk produced during the initial few days of lactation is called colostrum.
  - The colostrum contains several antibodies essential to develop resistance for newborn babies.

Know the Terms

- **Spermatogenesis :** It is the process of formation of sperms (spermatozoa) from the immature germ cells in males.
- **Spermiogenesis :** It is the process of transformation of non-motile, rounded haploid spermatid into a functional and motile spermatozoan.
- **Spermiation :** It is the process when mature spermatozoa are released from the sertoli cells into the lumen of seminiferous tubules.
- **Oogenesis :** It is the process of formation of a mature ovum from the oogonia in female.
- **Menstrual cycle :** The cyclic events starting from one menstruation till the next that take place during the reproductive period is called menstrual cycle.
- **Fertilization :** The process of fusion of sperm and the ovum to form a single cell called zygote is called fertilization.
- **Morula :** The embryo with 8-16 blastomeres is called a Morula.
- **Parturition :** The birth of the fully developed foetus is termed as parturition.
- **Colostrum :** The milk produced during the initial few days of lactation is called colostrum. It contains several antibodies absolutely essential to develop resistance for the new-born babies.
- **Foetal Ejection Reflex :** It is the initial and mild contractions of the uterus initiated by the fully developed foetus and the placental hormones.
- **Foetus :** An advanced stage of embryo within the uterus.
- **Gestation Period :** A period between fertilisation of ovum and the birth of a baby.
- **Hymen :** A thin membrane partially covering the vaginal aperture.
- **Insemination :** It refers to the deposition of sperms into the female genital tract.
- **Implantation :** It is the process in which the embryo becomes embedded into the wall of the uterus.
- **Lactation :** It refers to the feeding of the new born baby with mother's milk.
- **Menarche :** It is the beginning of menstruation at puberty in primate females.
- **Menopause :** It refers to the stoppage of menstruation at around the age of 45 – 50.

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CHAPTER-3
REPRODUCTIVE HEALTH

Revision Notes

- **Reproductive Health**
  - The term 'reproductive health' simply refers to healthy reproductive organs with normal functions. According to WHO (World Health Organisation), the word 'reproductive health' means a total well-being in all aspects of reproduction i.e., physical, emotional, behavioural and social.
  - Thus, a society with people having physically and functionally normal reproductive organs and normal emotional and behavioural interactions among them in all sex-related aspects might be called reproductively healthy.
- **Problems Associated with Reproductive Health :**
  (a) Rapid increase in the human population called population explosion.
  (b) Lack of awareness and sex education in people.
  (c) Several of myths and misconceptions about sex-related aspects.
  (d) Common occurrence of sexually transmitted diseases due to lack of knowledge of hygiene of reproductive organs.
  (e) Illegal abortions and female foeticides.
  (f) Sex abuse and sex-related crime.
- **Strategies of Reproductive Health Programmes :** To ensure total reproductive health, several programmes like reproductive health programmes and family planning were started in 1951.
- **The aims of Reproductive and Child Care Programmes :**
  (a) **Create awareness** in both males and females about various reproductive aspects with the help of audio-visual and print media by both Government and Non-Government agencies.
  (b) Provide **sex education** in schools to save the young generation from myths and misconceptions about sex related issues.

(c) **Prevention and control of sexually transmitted diseases** by providing the correct information about reproductive organs, adolescence and safe and hygienic sexual practices.

(d) **Educate the fertile couples** and those in marriageable age about birth control devices, pre-natal and post-natal care of mother and child, importance of breastfeeding, etc.

(e) Provide awareness about (ill-effects of population explosion, sexual abuses, sex discrimination and sex related crimes.)

(f) **Provide medical facilities and support** like infra structural facilities, professional expertise and material support to decrease maternal and infant mortality rates.

(g) Reduce the **problem of infertility** by promoting Assisted Reproductive Techniques (ARTs).

- **Steps Taken to Maintain a Reproductively Healthy Society**
  1. Imposing a statutory ban on amniocentesis (analysis of amniotic fluid-Foetal Sex Determination.)
  2. Rigorous implementation of immunization programs.
  3. Creation of specialized health centres like infertility clinic for diagnosis and corrective treatment of some infertility disorders.
  4. Better awareness about sex-related matters and sex-related problems, etc.
  5. Increase in the number of medically assisted deliveries and better post-natal care.
  6. Increase in the number of couples with small families.
  7. Early detection and cure of STDs.
- **Reasons for Population Explosion :** Tremendous increase in size and growth rate of population is called population explosion. It is due to :
  1. Rapid decline in death rate.
  2. More longevity, longer life span.
  3. Advanced medical facilities.
  4. Prevention of diseases.
  5. Developed techniques in agriculture.
  6. Better transport facilities.
  7. Protection from natural factors.
  8. Increase in the number of people of reproductive age.
- **Consequences of Population Explosion :** Poverty, unemployment, shortage of food, unhygienic conditions, education problems, residential problems, pollution, crime, excessive consumption of natural resources etc.
- **How to Control Over Population ?**
  1. People should be given education regarding the advantages of small family and family planning methods.
  2. Increasing the age of marriage.
  3. Incentives to those families, who are adopting family planning methods.
  4. Birth control through vasectomy and tubectomy.
  5. Family planning programmes with the slogan '**Hum Do Hamare Do**'.
- **Birth Control Measures :**

  The most important step to overcome this problem is to encourage smaller families by using various contraceptive methods. The contraceptive methods help to prevent unwanted pregnancies.
- **An ideal contraceptive should be**
  - User-friendly, easily available, effective and reversible.
  - With no or least side-effects.
  - Non-interfering with sexual drive, desire & sexual act.
- They are grouped as follows :

1. **Natural or Traditional Methods :** These methods of birth control depend upon the natural rhythm of a woman. These include the following methods **:**

   (a) **Coitus interruptus :** This involves withdrawing the penis by the male partner before ejaculation so that semen is not deposited in the vagina. It is the oldest method of voluntary fertility control. This method has certain limitations:
   - Some sperms may be deposited in the vagina even before the sexual climax.
   - May develop physiological and psychological problems for both partners.

   (b) **Periodic abstinence :** A week before and a week after the menstrual phase are supposed to be safe periods for sexual intercourse. It reduces the chances of pregnancy by about 80 percent.

   The period from 10^{th} to 17^{th} day (both days included) of the menstrual cycle is called danger or risk or fertile period and unprotected sexual intercourse should be avoided during this period.

   (c) **Lactational amenorrhea :** It has been noticed that during the period of intense lactation after the parturition, the mother does not undergo menstruation and ovulation so, the chances of conception are nearly nil. It also has no side effects but it is effective only up to a maximum period of six months after parturition.

2. **Artificial Methods :** This involves mechanical or barrier methods.

   (a) **Condoms :** These are rubber or latex sheaths that are put on the penis before coitus (copulation). These are

popularly called 'Nirodh'. These check pregnancy by preventing the deposition of sperms in the vagina. These can be self-inserted to give privacy to the user. These also prevent the spread of sexually transmitted diseases (STDs) including AIDS, syphilis, etc. Female condoms are also available called femidoms.

(b) Diaphragms and cervical caps : These are mechanical barriers made of rubber and fitted in the vagina of a female to check the entry of sperms in the uterus. These are reusable.

(c) Intra Uterine Devices (IUDs) : These are inserted by doctors or expert nurses in the uterus through the vagina. These include :

- Non-medicated IUDs (*e.g.,* Lippes loop)
- Copper releasing IUDs (*e.g.,* Copper T)
- Hormone releasing IUDs (e.g., Progestogen) : Make the uterus unsuitable for implantation and the cervix hostile to the sperms.
- IUDs increase the phagocytosis of sperms. The Cu^+ ions suppress the motility and fertilizing capacity of sperms.
- IUDs are ideal contraceptives for females who want to delay pregnancy or spacing in children.

3. Chemical Methods : These are of the following types :

(i) Spermicidal tablets, jellies, paste and creams introduced in the vagina before coital activity. These kill sperms. Common spermicidal chemicals used are lactic acid, citric acid, potassium permanganate, zinc sulphate etc.

(ii) Physiological (Oral) Devices : These are the hormonal preparation in the form of pills for females.

- The pills are usually small doses of progestogens or progestogen–estrogen combinations in the form of tablets (pills).
- Pills are taken daily for 21 days starting within the first five days of the menstrual cycle. After a gap of 7 days (during which menstruation occurs) it has to be repeated in the same pattern as long as the female desires to prevent conception.
- They inhibit ovulation and implantation as well as alter the quality of cervical mucus to prevent the entry of sperms.
- Pills are very effective with lesser side effects.
- **Saheli :** It is a new oral contraceptive for females. It was developed by the **Central Drug Research Institute** (CDRI) Lucknow. It contains a non-steroidal preparation. It is a 'once a week' pill with very few side effects and high contraceptive value.
- **Drawbacks of Oral Contraceptives :** Nausea, abdominal pain, breakthrough bleeding, irregular menstrual bleeding, breast cancer etc.

(iii) Injectables/Implants

- Progestogens alone or in combination with an oestrogen is used by females as injections or implants under the skin.
- Their mode of action is similar to that of pills and their effective periods are much longer. These are also effective within 72 hours of coitus. Thus it has been found to very effective as emergency contraceptives.

4. Sterilization or Surgical Methods : These methods block gamete transport and so prevent conception. These include the following measures :

(a) Male sterilization : It is a permanent method of birth control in which either testes are surgically removed, called castration, or cutting of the vas deferens, called **Vasectomy**. The vas deferens is exposed and cut through a small incision on the scrotum to prevent the passage of sperms.

(b) Female sterilization : Methods of female sterilization include :

(i) Ovariectomy involves surgical removal of ovaries.

(ii) Tubectomy involves cutting of fallopian tubes.

(iii) Tubal ligation involves blocking of fallopian tubes by an instrument called a laparoscope.

➢ **Medical Termination of Pregnancy (MTP)**

- Intentional or voluntary termination of pregnancy before full term is called MTP or induced abortion.
- 45 to 50 million MTPs are performed in a year all over the world (*i.e.* 1/5th of the total number of conceived pregnancies).
- MTP helps to decrease the population.
- Because of emotional, ethical, religious and social issues many countries have not legalised MTP.
- Government of India legalized MTP in 1971 with some strict conditions to check indiscriminate and illegal female foeticides which are reported to be high in India.

➢ **Importance of MTP**

- To avoid unwanted pregnancies due to casual intercourse or failure of the contraceptive used during coitus or rapes.

- Essential in cases where continuation of the pregnancy could be harmful to the mother or to the foetus or both.
- MTPs are safe during the first trimester, (up to 12 weeks of pregnancy). 2nd-trimester abortions are very risky.

➢ **Problems Related to MTPs**
- Majority of the MTPs are performed illegally.
- Misuse of amniocentesis (a foetal sex determination test based on the chromosomal pattern in the amniotic fluid).
- MTP for a female child causes sex imbalance in society.

➢ **Amniocentesis**
- It is a prenatal diagnostic method to determine the sex of the developing baby. This method has both positive and negative application. This method is legally banned in India.

(a) Positive application
- It helps to detect any genetically controlled congenital disease or any metabolic disorders in the foetus.

(b) Negative application
- People use this method for female foeticide, which causes sex imbalance in society.

➢ **Sexually Transmitted Diseases (STDs)**
- Diseases transmitted through sexual intercourse are called Sexually transmitted diseases (STDs)/Venereal diseases (VD) or Reproductive tract infections (RTI). *e.g.* Gonorrhoea, syphilis, genital herpes, chlamydiosis, genital warts, trichomoniasis, hepatitis-B and HIV leading to AIDS.
- **Hepatitis-B and HIV are also transmitted:**
  (a) By sharing of injection needles, surgical instruments, etc.
  (b) By transfusion of blood.
  (c) From infected mother to foetus.
- Except Hepatitis B, genital herpes, HIV and other diseases are completely curable if detected early and treated properly.
- **Early symptoms :** Itching, fluid discharge, slight pain, swellings, etc., in the genital region.
- Absence or less significant early symptoms and the social stigma deter the infected persons to consult a doctor. This leads to pelvic inflammatory diseases (PID), abortions, stillbirths, ectopic pregnancies, infertility, cancer of the reproductive tract, etc.
- All persons are vulnerable to STDs. These are very high among persons in the age group of 15-24 years.
- **Prevention :**
  (a) Avoid sex with unknown partners/multiple partners.
  (b) Always use condoms during coitus.
  (c) In case of doubt, go to a qualified doctor for early detection and get complete treatment.

➢ **Infertility**
- It is the inability of male or female to produce children.
- The reasons for this may be physical, congenital, diseases, drugs, immunological or even psychological.

➢ **Assisted Reproductive Technologies (ART)**

(1) ***In vitro*** **fertilization (IVF– test tube baby programme) :** In this method, ova from the wife/donor and sperms from the husband/donor are collected and are induced to form zygote under simulated conditions in the laboratory. This is followed by Embryo transfer (ET). It is of 2 types :
  (a) Zygote Intra Fallopian Transfer (ZIFT) : Transfer of zygote or early embryos (with up to 8 blastomeres) into the fallopian tube.
  (b) Intra Uterine Transfer (IUT) : Transfer of embryos with more than 8 blastomeres into the uterus. The embryo formed by in vivo fertilization (fertilization within the female) is also used for such transfer to assist those females who cannot conceive.

(2) Gamete Intra Fallopian Transfer (GIFT) : Transfer of an ovum from a donor into the fallopian tube of another female who cannot produce ovum, but can provide a suitable environment for fertilization and development.

(3) Intra Cytoplasmic Sperm Injection (ICSI) : A laboratory procedure in which a single sperm (from a male partner) is injected directly into an egg (from a female partner). Then the fertilized egg is implanted into the woman's uterus.

(4) Artificial Insemination (AI) technique:
- The semen collected from the husband or a healthy donor is artificially introduced into the vagina or the uterus (IUI– intra-uterine insemination) of the female.
- This technique is useful for the male partner having an inability to inseminate female or low sperm counts, etc.

(5) Surrogacy
- Here, a woman (surrogate mother) bears a child for a couple unable to produce children, because the wife is infertile or unable to carry.
- The surrogate is impregnated either through artificial insemination or through the implantation of an embryo produced by in vitro fertilization.

- **Problems of ART**
  - It requires high precision handling by specialized professionals and expensive instrumentation. Therefore, these facilities are available only in very few centres and are affordable to only a limited number of people.
  - Emotional, religious and social problems.
  - Legal adoption is one of the best methods for couples looking for parenthood.

Know the Terms

- **Medical Termination of Pregnancy :** The Intentional or voluntary termination of pregnancy before full term is known as MTP.
- **Amniocentesis :** It is a prenatal diagnostic technique that is used to determine the sex and metabolic disorders of the developing foetus in the mother's uterus through the observation of the chromosomal pattern.
- **Sexually transmitted diseases :** Diseases transmitted through sexual intercourse are called sexually transmitted diseases (STDs).
- **Infertility :** It is the inability of couple to produce baby even after unprotected intercourse.
- **Zygote Intra Fallopian Transfer (ZIFT) :** It involves transfer of zygote or early embryos (with up to 8 blastomeres) into fallopian tube.
- **Intra Uterine Transfer (IUT) :** It involves transfer of embryos with more than 8 blastomeres into the uterus.
- **Artificial insemination (AI) technique :** It is the method of transferring semen (sperm) collected from the husband or a healthy donor into the vagina or the uterus (IUI – intra-uterine insemination) of the recipient female.
- **IUD :** Intra Uterine Devices. These are the devices inserted in the uterus to achieve contraception.
- **Periodic Abstinence :** It is a natural method of contraception in which the couples avoid coitus from day 10 to 17 of the menstrual cycle.
- **Lactational Amenorrhoea :** It refers to the absence of menstruation during the period of intense lactation following parturition.
- **Gamete Intra Fallopian Transfer (GIFT) :** It is the method of transfer of an ovum from donor to another female who cannot produce it, but can provide suitable environment for fertilisation and its further development.

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UNIT-VII : GENETICS AND EVOLUTION

CHAPTER-4

PRINCIPLES OF INHERITANCE AND VARIATION

TOPIC-1
Mendelian Laws of Inheritance and Chromosomal Theory of Inheritance

Revision Notes

Mendel's Laws of Inheritance :

- **Hybridization Experiments on Garden Pea (*Pisum sativum*)**
  - Mendel selected 7 pairs of contrasting traits of true breeding pea varieties.

S. No.	Characters	Dominant	Recessive
1.	Height of the stem	Tall (T)	Dwarf (t)
2.	Colour of the flower	Violet/Red (R)	White (r)
3.	Position of the flower	Axial (A)	Terminal (a)
4.	Shape of pod	Full/Inflated (I)	Constricted (i)

5.	Colour of pod	Green (G)	Yellow (g)
6.	Shape of seed	Round (R)	Wrinkled (r)
7.	Colour of seed/cotyledons	Yellow (Y)	Green (y)

Inheritance of One Gene

- **Monohybrid Cross :**
  - A cross involving two plants differing in one pair of contrasting characters.
  - *e.g.* Mendel crossed tall and dwarf pea plants to study the inheritance of one gene.
- **Steps in Making a Cross of Pea :**
  - Selection of two pea plants with contrasting characters.
  - Removal of anthers (emasculation) of one plant to avoid self- pollination. This is a female parent now.
  - Collection of pollen grains from the other plant (male parent) and transfer to female parent pollination).
  - Collection of seeds and production of offspring.
  - Mendel made similar observations for other pairs of traits and proposed that factors were inherited from parent to offspring. Later these factors were called genes.
  - The F_1 generation (Tt) when self-pollinated, produces gametes T and t in equal proportion.
  - Mendel self-pollinated the F_2 plants.
  - He found that dwarf F_2 plants continued to generate dwarf plants in F_3 & F_4 generation.
  - He concluded that the genotype of the dwarf was homozygous- tt.
- **Monohybrid Phenotypic Ratio :** 3 Tall : 1 Dwarf = 3 : 1
- **Monohybrid Genotypic Ratio :**
  1 Homozygous tall (TT) : 2 Heterozygous tall (Tt) : 1 Homozygous dwarf (tt)
- **Back cross and Test cross**
  - Back cross : Crossing of F_1 hybrid with either of its parent.
  - Test cross : Crossing of an F_1 hybrid with its recessive parent (Test cross ratio=1:1). It is used to find out the unknown genotype. Mendel conducted a test cross to determine the F_2 genotype.

Mendel's Principles or Laws of Inheritance :

1. **Principle of Dominance**
   - Characters are controlled by discrete units called factors.
   - Factors occur in pairs.
   - In a dissimilar pair of factors or contrasting alleles *i.e.,* in heterozygous condition, only one member of the pair expresses its effect in the hybrid and is called dominant while the manifestation of the other is masked and is called recessive.
2. **Law of Segregation**
   This law states that allelic pairs separate or segregate during gamete formation and randomly unite at fertilization, thus homozygous parent produces similar gametes. Heterozygous parent produces two kinds of gametes, each having one allele in equal proportion.

   The Concept of Dominance
   - In heterozygotes, there are dominant and recessive alleles.
   - The normal (unmodified or functioning) allele of a gene produces a normal enzyme that is needed for the transformation of a substrate.
   - The modified allele is responsible for the production of
     (i) The normal/less efficient enzyme or
     (ii) A non-functional enzyme or
     (iii) No enzyme at all
   - **In the first case :** The modified allele will produce the same phenotype like the unmodified allele. It becomes dominant.
   - **In 2nd and 3rd cases :** The phenotype is dependent only on the functioning of the unmodified allele. Here, the modified allele becomes recessive.

Non-Mendelian Inheritance

(a) Incomplete Dominance

- It is an inheritance in which heterozygous offspring shows an intermediate character between two parental characteristics. *e.g.* Flower colour in Snapdragon (dog flower or *Antirrhinum* sp.) and *Mirabilis jalapa* (4'O clock plant).
- Here, phenotypic and genotypic ratios are the same.
- Phenotypic ratio = 1 Red : 2 Pink : 1 White
- Genotypic ratio = 1 (RR) : 2 (Rr) : 1(rr)
- This means that 'R' was not completely dominant over 'r'.

(b) Co-dominance

- It is the inheritance in which both alleles of a gene are expressed equally and independently in a hybrid *i.e.* both the alleles are dominant, *e.g.* ABO blood grouping in humans.
- ABO blood groups are controlled by the gene I.

- The gene (I) has three alleles I^A, I^B and i. However, a person can have any two of these three alleles. I^A and I^B both are dominant alleles while *i* is a recessive allele.
- The alleles I^A and I^B produce antigen A and antigen B respectively on the RBC surface while allele *i* doesn't produce any antigen.
- When I^A and I^B are present together they both express their types of surface antigen A and B. This is due to co-dominance.

(c) Multiple Allelism

- Here more than two alleles govern the same character.
- Since in an individual only two alleles are present, multiple alleles can be found only when population studies are made. *e.g.* ABO blood grouping (3 alleles : I^A, I^B & i). The skin colour and height of humans are also examples of multiple alleles.

(d) Pleiotropy

- Pleiotropy is the phenomenon in which one gene controls many traits. For example, the gene in pea plants that controls the round and wrinkled texture of seeds also influences the phenotypic expression of starch grain size.
- So, if the starch grain size is considered as the phenotype, then from this angle, the alleles show incomplete dominance.
- Therefore, dominance is not an autonomous feature of a gene or the product that it has information for. It depends as much on the gene product and the production of a particular phenotype.

Inheritance of Two Genes

➢ **Dihybrid Cross**

- A cross between two parents differing in two pairs of contrasting characters.
- Mendel made some dihybrid crosses *e.g.* Cross between the pea plants with round shaped and yellow coloured seeds (RRYY) and wrinkled shaped and green coloured seeds (rryy).
- On observing the F_2 generation, Mendel found that the yellow and green colour segregated in a 3:1 ratio.
- Round and wrinkled seed shape also segregated in a 3:1 ratio.
  Thus, the segregation of one pair of contrasting characters (Round and wrinkled shape) is independent of the segregation of another pair of contrasting character (yellow and green) colour and also that some new combinations of character appear in F_2 generation as the alleles get randomly rearranged in the offsprings at the time of fertilization.
- Dihybrid genotypic ratio: 1 : 2 : 2 : 4 : 1 : 2 : 1 : 2 : 1
  RRYY =1; RRYy =2; RrYY = 2; RrYy = 4; RRyy = 1; Rryy = 2; rrYY = 1; rrYy = 2; rryy = 1
- Dihybrid Phenotypic ratio :
  Round yellow 9 : Round green 3 : Wrinkled yellow 3 : Wrinkled green : 1, *i.e.* 9 : 3 : 3 : 1
  The ratio of 9 : 3 : 3 : 1 can be derived as a combination series of 3 yellow : 1 green, with 3 round : 1 wrinkled. *i.e.* (3 : 1), (3 : 1) = 9 : 3 : 3 : 1

3. Mendel's Law of Independent Assortment :

- It states that when more than one pair of characters are involved in a cross, the segregation of one pair of contrasting characters is independent of the segregation of other pair of contrasting characters and also that new recombinations of characters along with the parental type also appear in the F_2 generation.

➢ **Non-recognition of Mendel's work**

- **Mendel's work remained unrecognizable till 1900 because :**
  (a) Communication was not easy.
  (b) Non-recognition of Mendel as a scientist.
  (c) His mathematical approach was new and unacceptable.
  (d) He used statistical calculations which were beyond the comprehension of the biologists of his time.
  (e) Chromosomes, mitosis and meiosis were not known in Mendel's time.
  (f) The concept of genes (factors) as stable and discrete unit was not accepted. (Mendel could not explain the continuous variations seen in nature).
  (g) Mendel could not provide any physical proof for the existence of factors.
- In 1900, de Vries of Holland, Correns of Germany & Von Tschermak of Austria independently rediscovered Mendel's results and proclaimed their conclusions as Mendel's Laws of inheritance.

➢ **Chromosomal Theory (1902)**

- The chromosomal Theory was proposed independently by Walter Sutton and Theodore Boveri in 1902.
- Walter Sutton & Theodore Boveri proposed that the pairing and separation of a pair of chromosomes during meiosis lead to the segregation of pair of factors.
- Sutton united chromosomal segregation with Mendelian principles and called it as the Chromosomal Theory of Inheritance.
- **It states that :**
  (a) Chromosomes are vehicles of heredity *i.e.*, they are transmitted from parents to offspring.
  (b) Two identical chromosomes form a homologous pair. Genes are present in a linear fashion on chromosomes.
  (c) They segregate at the time of gamete formation.
  (d) Independent pairs segregate independently of each other.
  (e) Chromosomes are mutable.
  (f) Sex chromosomes determine the sex of an individual.

Parallelism between Genes (Mendelian factors) & Chromosomes :

- Mendelian factors as well as chromosomes are transferred from generation to generation.
- The chromosomes occurs in homologous pairs. The genes also occurs in pairs (allele pairs).
- Both chromosomes and genes segregate at the time of gamete formation in such a way that gametes receive only one chromosome & similarly one allele of each pair.

- Different pairs of chromosomes segregate independently of each other. Similarly, one pair of alleles segregates independently of another pair.
- Fusion of two (male & female) gametes brings about the diploid chromosome number as well as the allelic pairs in the offsprings.
- Thomas Hunt Morgan proved the Chromosomal Theory of Inheritance using fruit flies **(***Drosophila melanogaster***).**
- **He took fruit flies as a suitable material because :**
  (a) It breeds very quickly.
  (b) Short generation time (life cycle : 12-14 days).
  (c) Breeding can be done throughout the year.
  (d) Hundreds of progenies are produced per mating.
  (e) They can grow on a simple synthetic medium.
  (f) Male and female flies are easily distinguishable.

➢ **Linkage and Recombination**

- **Recombination :** It is a process by which pieces of DNA are broken and recombined to produce a new combination of alleles.
- **Linkage :** Physical association of two or more genes on a chromosome, which show the tendency to inherit together. They do not show independent assortment.
- Morgan et. al crossed yellow body and white eyed females with wild type brown body and red-eyed males and inter-crossed F_1 offsprings. He found that the two genes did not segregate independently, resulted in deviation from normal dihybrid ratio 9 : 3 : 3 : 1 in F_2 generation because the appearance of parental combinations were higher than the non-parental and new recombinations.
- Morgan further carried out several dihybrid test crosses in *Drosophila* to study sex-linked genes.
  Cross A : Double recessive, yellow-bodied, white-eyed females (YW/YW) X hybrid brown-bodied, red-eyed males (Y'W'/YW) (wild type).
  Cross B : Double recessive, white-eyed, miniature winged (Wm/Wm) X hybrid red eyed, large winged (W'm/Wm) (wild type).
- **Morgan in the above crosses found that :**
  (a) The two genes did not segregate independently of each other and the F_2 ratio deviated from the 9 : 3 : 3 : 1 ratio.
  (b) Genes were located on the X chromosome.
  (c) When two genes were situated on the same chromosome, the proportion of parental gene combinations was much higher than the non-parental type. This is due to linkage.
  (d) Genes for white and yellow were very tightly linked and showed only 1.3% new recombination while white and miniature wings showed 37.2% recombination (loosely linked).
  (e) Tightly linked genes show low recombination.
  (f) Loosely linked genes show high recombination.
  The strength of linkage is inversely proportional to the distance between two linked genes. Thus, the linkage between y & w alleles is stronger than the linkage between w & m alleles.
- **Linkage groups :** All the genes present together on a single chromosome make up a linkage group. The total number of linkage groups in an organism is equal to its haploid number of chromosomes or number of homologous pairs in diploid organisms.
- Alfred Sturtevant used the recombination frequency between gene pairs as a measure of the distance between genes and 'mapped' their position on the chromosome.
- Recombination frequency or the cross over value (COV) can be calculated by the following formula.

$$\text{COV} = \frac{\text{Number of recombinants}}{\text{Total number of offsprings}} \times 100$$

- Genetic maps are used as a starting point in the sequencing of genomes as was done in Human Genome Project.

TOPIC-2
Sex Determination and Chromosomal Disorder

Revision Notes

➢ **Sex determination**

- The method by which the distinction between male and female is established in a species is called sex determination.
- Sex of an individual is finalized at the time of zygote formation.

➢ **Autosomes and Sex chromosomes (allosomes)**

- Autosomes are chromosomes other than sex chromosomes. They contain genes that determine somatic characteristics.
- Number of autosomes is the same in males and females.
- Sex chromosomes (X & Y) are the chromosomes that are involved in sex determination.
- **Henking (1891)** studied spermatogenesis in some insects and observed that 50 % of sperm received a nuclear structure after spermatogenesis, whereas the other 50 % of sperms did not receive it.
- Henking called this structure as the **X body** (later it was called as **X-chromosome**).

➢ **Mechanism of Sex Determination**

(i) Chromosomal sex determination : It is based on heterogamety i.e., the occurrence of two types of gametes in one of the two sexes. It is of the following types :

(a) XX-XO mechanism :

Here, the male is heterogametic *i.e.* XO besides autosomes (gametes with X and gametes without X) and female is homogametic *i.e.* XX (all gametes are with X chromosomes) *e.g.* Many insects such as grasshopper.

(b) XX-XY mechanism :

Male is heterogametic (X & Y) and female is homogametic (X only). *e.g.* Human and *Drosophila.*

(c) ZZ-ZW mechanism :

Male is homogametic (ZZ) and female is heterogametic (Z & W). *e.g.* Birds.

(d) ZO-ZZ mechanism : Females have only Z-chromosomes besides autosomes and males have a pair of Z-chromosomes *e.g.* in cockroaches.

XX-XO & XX-XY mechanisms show male heterogamety. ZZ-ZW mechanism shows female heterogamety.

Females have only Z chromosome besides autosomes and males have a pair of Z chromosome as seen in cockroaches.

➢ **Sex Determination in Humans (XX-XY type)**

- Human has 23 pairs of chromosomes (22 pairs are autosomes and 1 pair is sex chromosome).
- A pair of X-chromosome (XX) is present in the female, whereas the X and Y chromosome are present in male.
- During spermatogenesis males produce 2 types of gametes *i.e.*, 50 % with X-chromosome and 50 % with Y-chromosome.
- Females produce the only ovum with X-chromosome.
- There is an equal probability of fertilization of the ovum with the sperm carrying either the X or Y chromosome.
- The sperm determines whether the offspring will be male or female.

(ii) Environmental Sex-determination : Determination of sex depends upon the environmental condition. Environmental factors like temperature etc. determine whether the zygote will develop into male or female. *e.g.* In turtles and crocodile.

(iii) Genetic balance mechanism of sex determination : The sex of the individual is decided by the ratio of X-chromosome and autosome as is found in ***Drosophila***.

(iv) Cytoplasmic Sex-determination : Cytoplasmic or fertility factor called as an F^+ factor located in plasmid determines the sex as is found in some bacteria.

➢ **Mutation**

- It is a sudden heritable change in DNA sequences resulting in changes in the genotype and the phenotype of an organism. The term mutation was given by Hugo de Vries (1901).
- It is caused either by loss or gain or change in a single base pair of DNA.
- **Frame-shift mutation :** Loss (deletions) or gain (insertion/ duplication) in DNA segment so that the whole frame of codons get changed.
- **Point mutation :** Mutation due to change in a single base pair of DNA. *e.g.* sickle cell anaemia.
- Mutation results in Chromosomal abnormalities (aberrations).
- Chromosomal aberrations are seen in **cancer cells**.
- **Mutagens** (agents which induce mutation) include,
  - **(a) Physical mutagens:** UV radiation, α, β, γ rays, X-ray, etc.
  - **(b) Chemical mutagens:** Mustard gas, phenol, formalin, etc.

➢ **Pedigree Analysis**

- The representation or chart showing family history is called the **family tree (pedigree).**
- Thus, analysis of traits in several generations of a family is called **pedigree analysis.**
- In humans, control crosses are not possible.
- So, the study of family history about inheritance is used.
- In human genetics, the pedigree study is utilized to trace the inheritance of a specific trait, abnormality or disease.

➢ **Genetic Disorders**

- There are two types of genetic disorders namely, Mendelian disorders and Chromosomal disorders.

(1) Mendelian Disorders

- It is caused by alteration or mutation in a single gene.
- The pattern of inheritance of Mendelian disorders can be traced in a family by the pedigree analysis.
  e.g. Haemophilia, Cystic fibrosis, Sickle-cell anaemia, Colour blindness, Phenylketonuria, Thalassemia, etc.
- Mendelian disorders may be dominant or recessive.
- By pedigree analysis one can easily understand whether the trait is dominant or recessive.

➢ **Pedigree Analysis of Autosomal Dominant Trait:** *e.g.* – Myotonic dystrophy

(a) Myotonic Dystrophy

- It is an autosomal dominant disorder that is characterized by increasing contractility of muscles with decreasing relaxation. This leads to atrophy of muscles, particularly of the face and neck. Hypogonadism, balding and cardiac irregularities may also be caused due to this disorder.

➢ **Pedigree Analysis of Autosomal Recessive Trait:** *e.g.* – Sickle cell anaemia

(a) Sickle-cell Anaemia

- This is an autosome linked recessive trait.
- It can be transmitted from parents to the offspring when both the partners are the carrier for the gene (or heterozygous).
- The disease is controlled by a pair of allele, Hb^A and Hb^S.
- Homozygous dominant (Hb^AHb^A) : normal; Heterozygous (Hb^AHb^S): carrier of sickle cell trait; Homozygous recessive (Hb^SHb^S) : affected
- The defect is caused by the substitution of Glutamic acid (Glu) by Valine (Val) at the sixth position of the β-globin chain of the haemoglobin (Hb).
- This is due to the single base substitution at the sixth codon of the β-globin gene from GAG to GUG.
- The mutant Hb molecule undergoes polymerization under low oxygen tension causing the change in the shape of the RBC from biconcave disc to elongated sickle-like structure.

(b) Haemophilia (Royal disease)

- Sex-linked recessive disease.
- In this, a protein involved in the blood clotting is affected.
- A simple cut results in non-stop bleeding.
- The heterozygous female (carrier) for haemophilia may transmit the disease to sons.
- The possibility of a female becoming a haemophilic is very rare because mother has to be at least carrier and the father should be haemophilic (inviable in the later stage of life).
- Queen Victoria was a carrier of the disease. So her family pedigree shows many haemophilic descendents.

(c) Phenylketonuria

- An inborn error of metabolism.
- Autosomal recessive trait.
- The affected individual lacks an enzyme (phenylalanine hydroxylase) that converts the amino acid phenylalanine into tyrosine.
- As a result, phenylalanine accumulates and converts into phenyl pyruvic acid and other derivatives.
- They accumulate in the brain resulting in mental retardation.
- These are also excreted through urine because of poor absorption by kidney.

(2) Chromosomal Disorders

- They are caused due to the absence or excess or abnormal arrangement of one or more chromosomes.
- These are of two types namely,
  (a) Aneuploidy **(b)** Euploidy.

(a) Aneuploidy

- The gain or loss of chromosomes due to failure of segregation of chromatids during cell division.
  It includes,
  (a) Nullisomy (2n-2) : A complete homologous pair is lost from diploid set.
  (b) Monosomy (2n-1) : One chromosome is lost from the diploid set.
  (c) Trisomy (2n+1) : One chromosome is added to the diploid set, so that one chromosome occurs in triplicate.
  (d) Tetrasomy (2n+2) : Two chromosomes are added to the diploid set, so that a chromosome is found in quadrupled.

(b) Polyploidy (Euploidy)

- It is an increase in the number of chromosomes sets beyond the diploid X condition (2n).
- This is often seen in plants.
- Based on the number of chromosome sets, the polyploid are of the following types : triploids (3n), tetraploids (4n), pentaploids (5n), hexaploids (6n), etc.
  (a) Autopolyploidy : It is an increase in number of the same genome. *e.g.* AAA (autotriploid), AAAA (autotetraploid), etc.

(b) Allopolyploidy : It is the increase in the number of sets of chromosome due to the coming together of diploid genomes of two or more than two individuals of different species. *e.g*. AABB, AABBDD. Bread wheat is allohexaploid (AABBDD). Triticale is the man-made cereal formed by hybridization between durum, wheat and rye. It is allohexaploid.

- **Autoallopolyploidy :** It is a kind of polyploidy where the genomes of two species come together in which one has double set of chromosomes. *e.g. Helianthus tuberosus* which is autoallohexaploid.
- **Chromosomal aberrations :** These are the changes in morphology and structure of chromosome resulting in the change in number and sequence of genes on them without any change in ploidy. They are of the following types :
  1. **Deletion :** It is the loss of a terminal segment of a chromosome or from within the chromosome (interstitial segment) followed by a reunion of its remaining parts.
  2. **Inversion :** It is a change in a chromosome architecture due to breaking up, rotation through 180° of a segment and its reunion so that sequence of genes is reversed in the inverted region.
  3. **Duplication :** It is a change in chromosome structure in which a part of a chromosome breaks up and unites with another homologous chromosome. This process repeats the chromosome segments because the same block of genes is present more than once in a haploid component.
  4. **Translocation :** It is a change in chromosome architecture that is due to breaking up of segment of chromosome and its union with another non-homologous chromosome. It may also be due to mutual exchange of chromosomal segments between non-homologous chromosomes.

➢ **Examples for Chromosomal Disorders**

(a) Down's Syndrome (Mongolism) :

- It is the presence of an additional copy of chromosome number 21 (trisomy of 21).
- Genetic constitution : 45 A + XX or 45 A + XY (*i.e*. 47 chromosomes).
- **Features :**
  - **(a)** They are short-statured with small round head.
  - **(b)** Broad flat face.
  - **(c)** Furrowed big tongue and partially open mouth.
  - **(d)** Many "loops" on fingertips.
  - **(e)** Palm is broad with characteristic palm crease.
  - **(f)** Retarded physical, psychomotor & mental development.
  - **(g)** Congenital heart disease.

(b) Klinefelter's Syndrome :

- It is the presence of an additional copy of X-chromosome in male.
- Genetic constitution: 44 A + XXY (*i.e*. 47 chromosomes).
- **Features :**
  - **(a)** Overall masculine development however the feminine development is also expressed. *e.g*. development of breast (Gynaecomastia).
  - **(b)** Sterile.
  - **(c)** Mentally retarded.

(c) Turner's Syndrome :

- This is due to the absence of one of the X chromosomes in female.
- Genetic constitution: 44 A + XO (*i.e.* 45 chromosomes).
- **Features :**
  - **(a)** Sterile, Ovaries are rudimentary.
  - **(b)** Lack of other secondary sexual characters.
  - **(c)** Dwarf.
  - **(d)** Mentally retarded.

Know the Terms

➢ **Alleles or allelomorphs :** A pair of Mendelian factors or genes located on the same locus of two homologous chromosomes of an individual which control the expression of a trait or character are called alleles or allelomorphs.

➢ **Back cross :** Cross between hybrid and one of its parent.

➢ **Clone :** The group of organisms produced by asexual reproduction. They are morphologically and genetically similar to one another as well as their parents (The individuals of a clone are called Ramet).

➢ **Cross :** Deliberate mating of two parental types of organisms of the same species.

- **Diploid :** An individual or cell containing two complete sets of chromosomes.
- **Diplotene chromosomes :** Lampbrush Chromosomes.
- **Dominant factor or allele :** It is one of a pair of alleles which can express itself whether present in homozygous or heterozygous state. e.g. T (tallness in pea), R (round seed in pea), A (axial flower in pea).
- **Dysgenics :** Study of undesirable traits of human race and the genes that cause them.
- **F_1 generation :** Hybrids Produced from a cross between the genetically different individuals called parents. e.g. Tt individuals are produced in F_1 generation from a cross between TT and tt parents.
- **F_2 generation :** It is the generation of individuals which arises as a result of interbreeding or selfing amongst individuals of F_1 generation.
- **Father of genetics :** Gregor Johann Mendel
- **Felix Bernstein :** Discovered multiple alleles, co-dominance and dominant recessive relationship in determination of human blood groups.
- **Gene locus :** A particular portion or region of the chromosomes representing a single gene is called gene locus.
- **Gene pool :** Aggregate of all the genes and their alleles present in an interbreeding population is known as gene pool.
- **Genetics (Gk. Genesis – origin) :** It is a branch of biology that deals with the study of principles and mechanism of heredity and variations. The term genetics was coined by Bateson (1906).
- **Genome :** It is a complete set of chromosomes where every gene/chromosome is represented singly as in a gamete.
- **Genotype : (Gk. Geno- race; typos – image).** It is the genetic constitution of individual with regard to one or more characters irrespective that whether the genes are expressed or not, for e.g. genotype of hybrid tall pea plant is Tt, pure tall TT and pure dwarf tt.
- **Haploid (Monoploid) :** An individual or cell containing a single complete set of chromosomes.
- **Heredity (L. hereditas – heirship or inheritance) :** It is the sum of all biological processes by which a particular characteristics are passed on from parents to their offspring, either through asexual reproduction or sexual reproduction.
- **Heterozygote (heterozygous) :** It is an individual which contain the two contrasting factors of a character or two different alleles of a gene on the same locus of its homologous chromosomes. It is not pure and is called hybrid for that character. e.g. Tt.
- **Homozygote (homozygous) :** It is an individual which contains identical alleles of a gene or factor of a character on its homologous chromosomes. e.g. TT or tt.
- **Hybrid :** The organisms produced after crossing two genetically different individuals is called hybrid. Also called heterozygote or heterozygous individuals.
- **Inheritance :** Transmission of characters from parents to progeny.
- **Ishihara cards :** Cards used for checking colour blindness.
- **Mendelian factor or gene :** It is a unit of inheritance which passes from one generation to the next through the gamete and controls the expression of a character in the organisms.
- **Phenotype :** (Gk. Pheno – to appear, typos – image): It is observable or measurable distinctive structural or functional characteristic of an individual. e.g. phenotypic tall pea plant can be genotypically TT ot Tt.
- **Punnett square (Checker board) :** A grid that enables to calculate the results of simple genetic crosses.
- **Recessive factors or allele :** The factor of an allelic or allelomorphic pair which is unable to express its effect in the presence of its contrasting factor in a heterozygote is called recessive factor or allele. The effect of recessive factor becomes known only when it is present in the pure or homozygous state, e.g. tt in dwarf pea plant.
- **Reciprocal cross :** Cross which involves two types of individual where the male of one type is crossed with female of the second type and vice versa.
- **Test cross :** Cross to know whether an individual is homozygous or heterozygous for dominant characters. The F_1 individual is crossed with one of its recessive parent.
- **Trait :** A phenotypic characteristic of an inherited character.
- **Variation :** Tendency of differences in various traits of individuals of a progeny from one another and their parents.
- **W. Johannsen :** Coined the term pure line (1903), gene (1909), genotype and phenotype.
- **Wild and mutant alleles (wild and mutant phenotype) :** Wild allele is one which is originally present in the population and is dominant, usually widespread. Mutant allele is less common and is believed to be formed through mutation of wild allele.
- **Wild type :** The species variety most commonly found in the natural population.

❑❑

CHAPTER-5
MOLECULAR BASIS OF INHERITANCE

TOPIC-1
Nucleic Acid – DNA and RNA

Genetic Material

- **Nucleic Acids**
  - DNA and RNA are the two types of nucleic acids.
  - DNA is the genetic material in all organisms except some viruses.
  - RNA is the genetic material in some viruses.
  - RNA mostly functions as messengers.
- **Structure of Polynucleotide Chain**
  - Polynucleotides are the polymers of nucleotides.
  - DNA and RNA are examples of polynucleotides.
  - **A nucleotide has 3 components :**
    1. A nitrogenous base
    2. A pentose sugar (ribose in RNA and deoxyribose in DNA)
    3. A phosphate group
  - Nitrogen bases are of two types :
    (a) Purines : It includes Adenine (A) and Guanine (G).
    (b) Pyrimidines : It includes Cytosine (C), Thymine (T) and Uracil (U). Thymine (5-methyl Uracil) present only in DNA and Uracil only in RNA (In place of thymine).
  - A nitrogenous base is linked to the pentose sugar through an N-glycosidic linkage to form nucleoside.

Nucleosides in RNA	Nucleosides in DNA
Adenosine	*Deoxyadenosine*
Guanosine	*Deoxyguanosine*
Cytidine	*Deoxycytidine*
Uridine	*Deoxythymidine*

  - Nitrogen base + sugar + phosphate group = Nucleotide (deoxyribonucleotide). In RNA, every nucleotide residue has an additional – OH group present at 2′-position in the ribose.
  - 2 nucleotides are linked through **3′ – 5′ phosphodiester** bond to form dinucleotide.
  - When series of nucleotides are linked together, it forms polynucleotide.
- **Structure of DNA**
  - **Johann Friedrich Miescher (1869) :** Identified DNA and named it as 'Nuclein'.
  - **James Watson & Francis Crick** proposed the double helix model of DNA. It was based on the X-ray diffraction data produced by **Maurice Wilkins & Rosalind Franklin**.
  - DNA is made of two polynucleotide chains coiled in a right-handed fashion. Its backbone is formed of sugar and phosphates. The bases project inside.
  - The two chains have anti-parallel polarity *i.e.* one chain has the polarity 5′ → 3′ and the other has 3′ → 5′.
  - Nitrogen bases of opposite chains are held together by hydrogen bonds forming base pairs (bp).
  - There are two hydrogen bonds between A and T (A = T) and three H-bonds between C and G (C ≡ G).
  - Purine comes opposite to a pyrimidine. This generates a uniform distance between the two strands.
- **Erwin Chargaff's Rule**
  - Purines and pyrimidines are always in equal amounts *i.e.* A + G = T + C.
  - In DNA, the proportion of A is equal to T and the proportion of G is equal to C *i.e.* A = T and G = C.
  - The base ratio A + T/G + C may vary from species to species but constant for a given species.
  - Length of DNA = number of base pairs × distance between two adjacent base pairs.

- Φ 174 (a bacteriophage) has 5386 nucleotides.
- Bacteriophage lambda has 48502 base pairs (bp).
- *E. coli* has 4.6×10^6 bp.
- Haploid content of human DNA = 3.3×10^9 bp.
- Number of base pairs in human = 6.6×10^9
- Length of DNA in humans = 6.6×10^9 bp × 0.34×10^{-9} m/bp = 2.2 m
- Length of DNA in *E. coli* =1.36 mm (1.36×10^{-3} m).

  ∴ The number of base pairs = 1.36×10^{-3} m/0.34×10^{-9} m/bp = 4×10^6 bp.

➢ **Packaging of DNA Helix**

- In prokaryotes (*e.g. E. coli*), the DNA molecule is held with some positively charged non-histone basic proteins like negatively charged polyamines and form 'nucleoid'.
- In eukaryotes, there is a set of positively charged basic proteins called histones.
- Histones proteins are rich in positively charged basic amino acid residues lysine and arginine.
- There are five types of histones proteins-H1, H2A, H2B, H3 and H4.
- Two molecules each of H2A, H2B, H3 and H4 organize to form a unit of eight molecules called as histone octamer.
- Negatively charged DNA is wrapped around positively charged histone octamer to form a structure called a nucleosome.
- Nucleosomes are connected with the help of linker DNA on which H1 Histone is present.

➢ **Nucleosome**

- A typical nucleosome contains 200 bp of DNA helix.
- Therefore, the total number of nucleosomes in human = 6.6×10^9 bp/200 bp = 3.3×10^7.
- Nucleosomes constitute the repeated unit to form chromatin.
- Chromatin is the thread-like stained bodies.
- Nucleosomes in chromatin appears as "beads-on-string" when it is viewed under the electron microscope..
- Chromatin is packaged to form a solenoid structure.
- Further supercoiling constitute a looped structure called chromatin fibre.

  These chromatin fibers further coil and condense at the metaphase stage of cell division to form chromosomes.
- Chromatin is packaged → solenoid → chromatin fibres → coiled and condensed at metaphase stage → chromosomes.
- Higher level packaging of chromatin requires non-histone chromosomal (NHC) proteins.
- Two types of chromatin are :
  - **(a) Euchromatin :** Loosely packed and transcriptionally active chromatin and stains light.
  - **(b) Heterochromatin :** Densely packed and inactive region of chromatin and stains dark.

➢ **The Search for Genetic Material**

Griffith's Experiment - Transforming Principle

- **Griffith** (1928) used mice and a bacterial strain, *Streptococcus pneumoniae*.
- *Streptococcus pneumoniae* has two strains :
  - **(a) Smooth (S) strain (Virulent) :** Has polysaccharide mucous coat that causes pneumonia.
  - **(b) Rough (R) strain (Non-virulent) :** No mucous coat that does not cause pneumonia.

➢ **Experiment**

- S-strain → Inject into mice → Mice die
- R-strain → Inject into mice → Mice live
- S-strain (Hk) → Inject into mice → Mice live
- S-strain (Hk) + R-strain (live) → Inject into mice → Mice die
- He concluded that there exists some 'transforming principle', that is transferred from heat-killed S-strain to R-strain. It enabled R-strain to synthesize smooth polysaccharide coat and become virulent. This must be due to the transfer of genetic material.

➢ **Biochemical Characterization of Transforming Principle**

- Oswald Avery, Colin MacLeod & Maclyn McCarty in 1944 worked to determine the biochemical nature of 'transforming principle' in Griffith's experiment.
- They purified biochemicals (proteins, DNA, RNA, etc.) from heat-killed S cells using suitable enzymes.
- They discovered that —
  - **(a)** Digestion of protein and RNA (using Proteases and RNases) did not affect transformation. So the transforming substance was not a protein or RNA.

(b) Digestion of DNA with DNase inhibited transformation. It means that DNA caused the transformation of R cells to S cells i.e. DNA was the transforming substance.

- **The Genetic Material is DNA**
  - The fact that DNA is the genetic material also came from the experiments of **Alfred Hershey** and **Martha Chase (1952)**.
  - They worked with viruses that infect bacteria and are called bacteriophages.
- **Hershey-Chase Experiment—Blender Experiment**
  - Hershey and Chase made two preparations of bacteriophage - In one, proteins were labelled with ^{35}S by putting in a medium containing radioactive sulphur (^{35}S). In the second, DNA was labelled with ^{32}P by putting in a medium containing radioactive Phosphorous (^{32}P).
  - These preparations were used separately to infect *E. coli*.
  - After infection, the *E. coli* cells were gently agitated in a blender to separate the phage particles from the bacteria.
  - Then the culture was centrifuged. Heavier bacterial cells were formed as a pellet at the bottom. Lighter viral components outside the bacterial cells remained in the supernatant.
  - They found that,
    (a) Supernatant contains viral protein labelled with ^{35}S, *i.e.* the viral protein had not entered the bacterial cells.
    (b) The bacterial pellet contains radioactive ^{32}P. This shows that viral DNA labelled with ^{32}P had entered the bacterial cells. This proves that DNA is the genetic material.
- **Properties of Genetic Material**
  - A molecule that can act as a genetic material must fulfill the following criteria :
    (a) Be able to generate its replica by the process of Replication.
    (b) Chemically and structurally be stable.
    (c) Allows slow changes, the mutations that are required for evolution.
    (d) It should be able to store genetic information which can be inherited.
    (e) Be able to express itself as 'Mendelian Characters'.
- **DNA is a Better Genetic Material than RNA due to the following reasons :**
  - DNA is chemically less reactive and structurally more stable. It can to undergo repair.
  - Due to the unstable nature of RNA, RNA viruses (*e.g. Qβ bacteriophage, Tobacco Mosaic Virus, etc.*) mutate and evolve faster.
  - For the storage of genetic information DNA is better due to its stability. But for the transmission of genetic information, RNA is better.
  - RNA can directly code for protein synthesis, hence can easily express the characters. DNA is dependent on RNA for protein synthesis.

Reasons for stability (less reactivity) of DNA	Reasons for mutability (high reactivity) of RNA
Double-stranded	*Single-stranded*
Presence of thymine	*Presence of Uracil*
Absence of 2'-OH	*Presence of 2'-OH*

  - The two DNA strands are complementary. On heating, they separate. When appropriate conditions are provided they come together. (In Griffith's experiment, when the bacteria were heat-killed, some properties of DNA did not destroy).
- **RNA World**
  - RNA is a single-stranded structure but it is often folded back upon itself forming helices. Nitrogenous bases are like those of DNA except that there is **uracil** in place of **thymine**.
  - RNA was the first regulatory chemical and genetic material in early life forms.
  - It acts as genetic material and biocatalyst.
  - Essential life processes (metabolism, translation, splicing, etc) evolved around RNA.
  - DNA has evolved from RNA with chemical modifications that made it more stable.
- **Central Dogma of Molecular Biology**
  - It was proposed by Francis Crick (1958). It states that the genetic information flows unidirectionally from DNA → RNA → Protein.
- **Reverse Transcription :** H. Temin and Baltimore in 1978 gave the concept of reverse flow of genetic information *i.e.* the formation of DNA from RNA. This is called Reverse Central Dogma or Teminism or reverse transcription. This takes place in some of the viruses in the presence of an enzyme called reverse transcriptase.
- **Types of RNA**
  - RNA is of 3 types –mRNA, tRNA and rRNA.
  - mRNA constitutes 2–5% of the total cellular RNA, tRNA is about 15% and rRNA is about 70–80%.

- **mRNA (messenger RNA) :** Provides a template for translation (protein synthesis) and is transcribed from DNA.
- **rRNA (ribosomal RNA) :** Structural and catalytic role during translation. *e.g.* ^{23}S rRNA in bacteria acts as ribozyme.
  It is the component of ribosome and is the most stable type of RNA.
- **tRNA (transfer RNA or sRNA or soluble RNA or adaptor RNA) :** Brings amino acids for protein synthesis and reads the genetic code.
- tRNA are smallest amongst all the RNA and is made up of 70–80 nucleotides only.

➢ **DNA Replication**
- Replication is the copying of DNA from parental DNA.
- **Watson** & **Crick** proposed a semi-conservative mode of replication.
- It suggests that the parental DNA strands act as a template for the synthesis of new complementary strands. After the completion of replication, each DNA molecule would have one parental and one new strand.

➢ **Experimental Proof**
- **Mathew Meselson** & **Franklin Stahl (1958)** experimentally proved semi-conservative mode.
- **Meselson & Stahl's Experiment :** They cultured *E. coli* in a medium containing $N^{15}H_4Cl$ (N^{15} : heavy isotope of N). N^{15} was incorporated into both strands of bacterial DNA and the DNA became heavier.
- Another preparation containing N salts labelled with N^{14} was also made. N^{14} was also incorporated in both strands of DNA and became lighter.
- These two types of DNA can be separated by centrifugation in a CsCl density gradient.
- They took *E. coli* cells from the N^{15} medium and transferred them to the N^{14} medium.
- After one generation (*i.e.* after 20 minutes), they isolated and centrifuged the DNA. Its density was intermediate (hybrid) between ^{15}N DNA and ^{14}N DNA. This showed that in the newly formed DNA, one strand is old (N^{15} type) and one strand is new (N^{14} type). This confirms the semi-conservative mode of replication.
- After II generations (*i.e.* after 40 minutes), there were equal amounts of hybrid DNA and light DNA.
- **Taylor et. al (1958)** performed similar experiments on *Vicia faba* (faba beans) using radioactive thymidine to detect distribution of newly synthesized DNA in the chromosomes. It proved that the DNA in chromosomes also replicate semi-conservatively.

➢ **The Machinery and Enzymes for Replication**
- DNA replication starts at a point called *origin* (*ori*).
- A unit of replication with one origin is called a *replicon*.
- During replication, the two strands unwind and separate by breaking H-bonds in the presence of an enzyme, *Helicase*.
- Unwinding of the DNA molecule at a point forms a 'Y'-shaped structure called replication fork.
- The separated strands act as templates for the synthesis of new strands.
- DNA replicates in the 5′→3′ direction.
- *Deoxyribonucleoside triphosphates* (dATP, dGTP, dCTP & dTTP) act as substrate and also provide energy for polymerization.
- Firstly, a small RNA primer is synthesized in presence of an enzyme, *primase*.
- In the presence of an enzyme, DNA dependent *DNA polymerase*, many nucleotides join with one another to primer strand and form a polynucleotide chain (new strand).
- The DNA polymerase forms one new strand (leading strand) on a continuous stretch in the 3′→5′ direction (Continuous synthesis).
- The other new strand is formed in small stretches (Okazaki fragments) in the 5′→3′ direction (Discontinuous synthesis).
- The Okazaki fragments are then joined together to form a new strand by an enzyme, *DNA ligase*. This new strand is called lagging strand.
- If a wrong base is introduced in the new strand, DNA polymerase can do proofreading.
- *E. coli* completes replication within 38 minutes *i.e.* 2000 bp per second.
- In eukaryotes, the replication of DNA takes place at the S-phase of the cell cycle. Failure in cell division after DNA replication results in polyploidy.

➢ **Transcription**
- It is the process of copying genetic information from one strand of the DNA into RNA.
- Here, adenine pairs with uracil instead of thymine.
- Both strands are not copied during transcription, because
  (a) The code for protein is different in both strands. This complicates the translation.
  (b) If two RNA molecules are produced simultaneously they would be complementary to each other, hence form a double-stranded RNA. This prevents translation.

- **Transcription Unit**
  - It is the segment of DNA between the sites of initiation and termination of transcription.
  - It consists of 3 regions :
    - **(a) A promoter (Transcription start site) :** Binding site for RNA polymerase.
    - **(b) Structural gene :** The region between promoter and terminator where transcription takes place.
    - **(c) A terminator :** The site where transcription stops.
  - The DNA- dependent RNA polymerase catalyses the polymerization only in 5′→3′direction.
  - 3′→5′ acts as the template strand. 5′→3′ acts as the coding strand.
  - 3′–ATGCATGCATGCATGCATGCATGC–5′ template strand.
    5′–TACGTACGTACGTACGTACGTACG–3′ coding strand.
- **Transcription Unit and the Gene**
  - **Gene :** Functional unit of inheritance. It is the DNA sequence coding for RNA molecule.
  - **Cistron :** A segment of DNA coding for a polypeptide.
  - Structural gene in a transcription unit is of two types :
    - **(a) Monocistronic structural genes (split genes) :** It is seen in eukaryotes. Here, the coding sequences (expressed sequences or exons) are interrupted by introns (intervening sequences).
    - **(b) Polycistronic structural genes :** It is seen in prokaryotes. Here, there are no split genes.
  - **Exons and Introns :** In eukaryotes, the monocistronic structural genes have interrupted coding sequences *i.e.* the genes in eukaryotes are split. The coding sequences or expressed sequences are called as **exons**. Exons are said to be those sequences that appear in mature or processed RNA. The exons are interrupted by **introns**. Introns or intervening sequences do not appear in mature or processed RNA.
- **Steps of transcription in prokaryotes**
  - **Initiation :** Here, the enzyme *RNA polymerase* binds at the promoter site of DNA. This causes the local unwinding of the DNA double helix. An initiation factor (σ factor) present in RNA polymerase initiates the RNA synthesis.
  - **Elongation :** The RNA chain is synthesized in the 5′-3′ direction. In this process, activated ribonucleoside triphosphates (ATP, GTP, UTP & CTP) are added. This is complementary to the base sequence in the DNA template.
  - **Termination :** A termination factor (ρ factor) binds to the RNA polymerase and terminates the transcription.
  - In bacteria (Prokaryotes), transcription and translation can be coupled (Translation can begin before mRNA is fully transcribed) because mRNA requires no processing to become active.
  - Transcription and translation take place in the same compartment (no separation of cytosol and nucleus).
- **In eukaryotes, there are 2 additional complexities :**
  - **(a) There are three RNA polymerases :**
    - **RNA polymerase I :** Transcribes rRNAs (28S, 18S & 5.8S).
    - **RNA polymerase II :** Transcribes the heterogeneous nuclear RNA (hnRNA). It is the precursor of mRNA.
    - **RNA polymerase III :** Transcribes tRNA, 5S rRNA and snRNAs (small nuclear RNAs).
  - **(b) The primary transcripts (hnRNA) :** They contain both the exons and introns and are non-functional. Hence introns have to be removed. For this, it undergoes the following processes :
    - **Splicing :** From hnRNA, introns are removed (by the spliceosome) and exons are spliced (joined) together.
    - **Capping :** Here, a nucleotide methyl guanosine triphosphate (cap) is added to the 5′ end of hnRNA.
    - **Tailing (Polyadenylation) :** Here, adenylate residues (200-300) are added at 3′-end. It is the fully processed hnRNA, now called mRNA.

IMPORTANT DIAGRAMS :

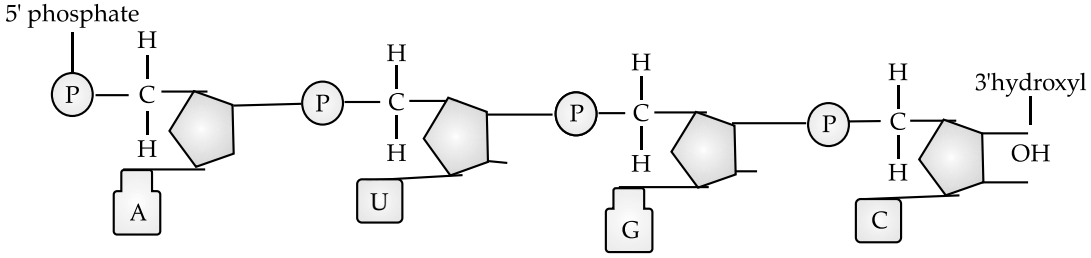

Fig 6.1 : A polynucleotide Chain of RNA

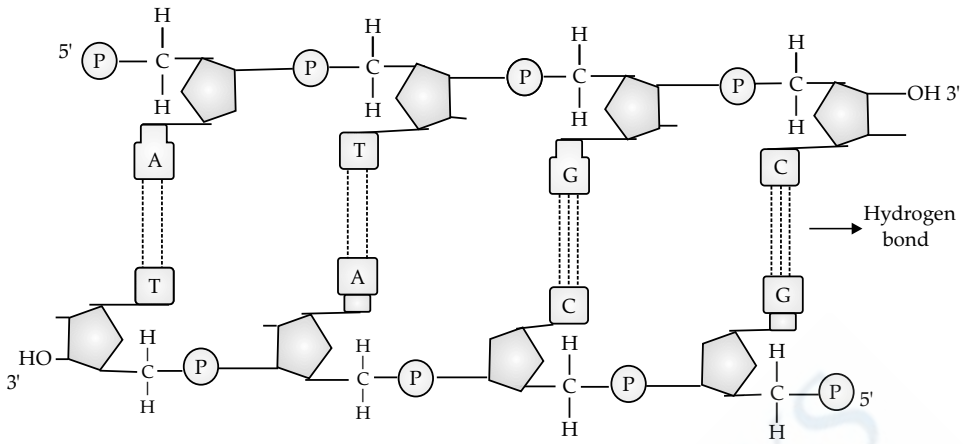

Fig 6.2 : Double Stranded polynucleotide chain

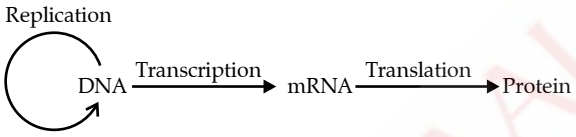

Fig 6.3 : Central Dogma

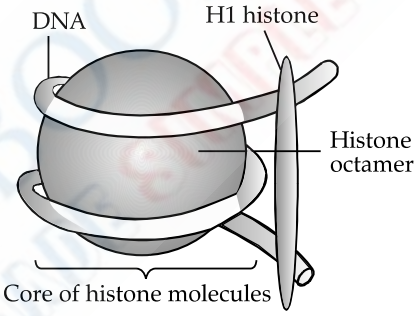

Fig 6.4 : Nucleosome

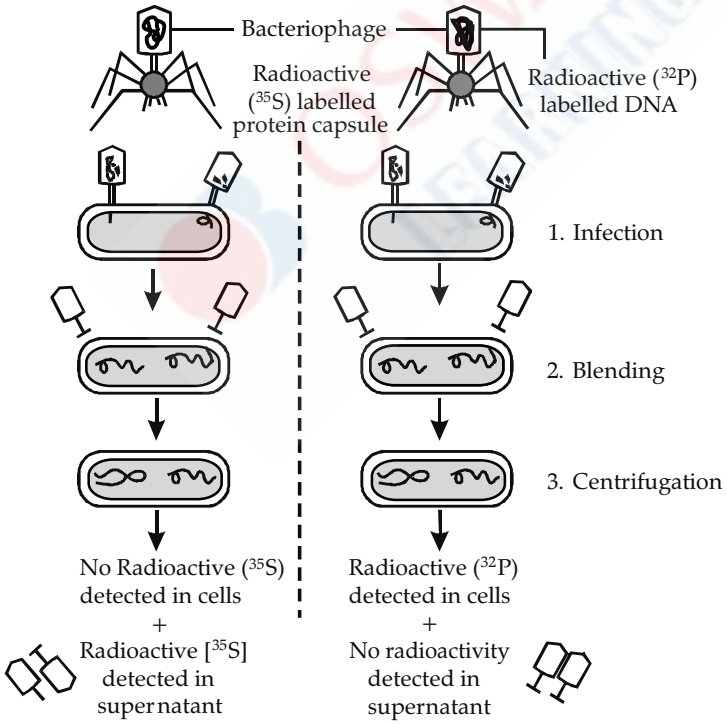

Fig 6.5 : The Hershey and Chase Experiment

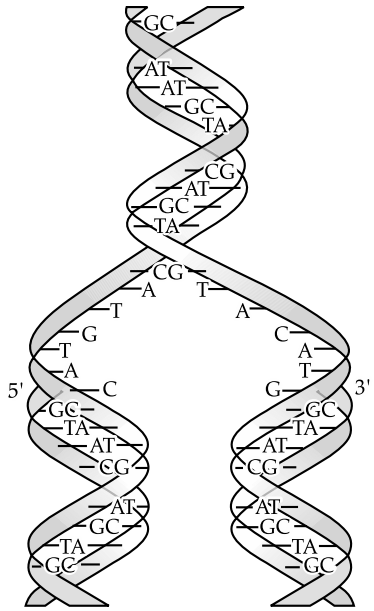

Fig 6.6 : Watson Crick model of Semi-conservative DNA replication

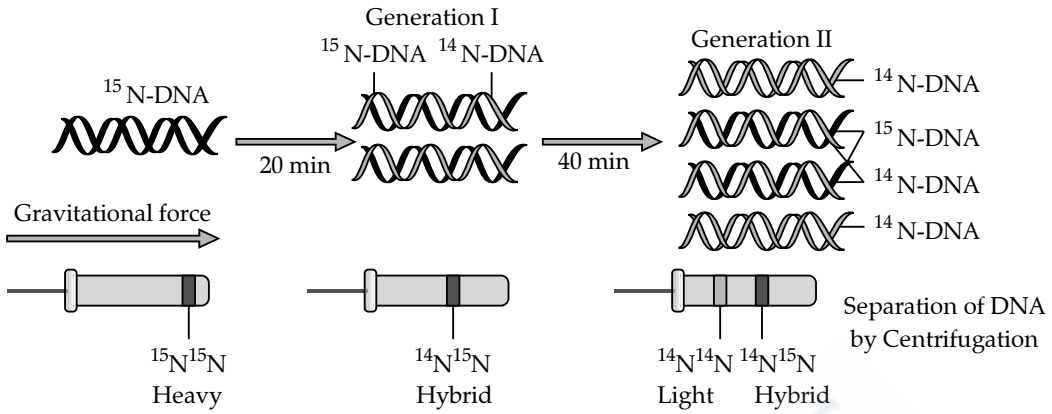

Fig 6.7 : Meselson and Stahl's Experiment

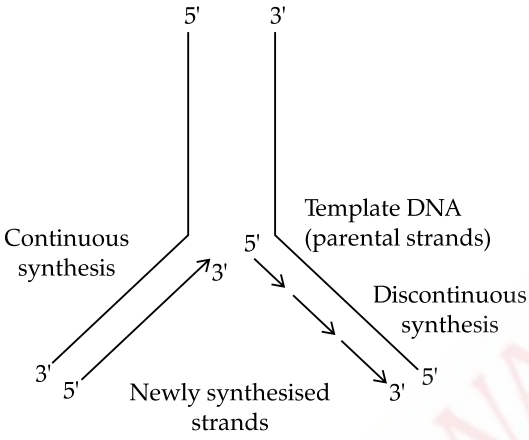

Fig 6.8 : Replicating Fork

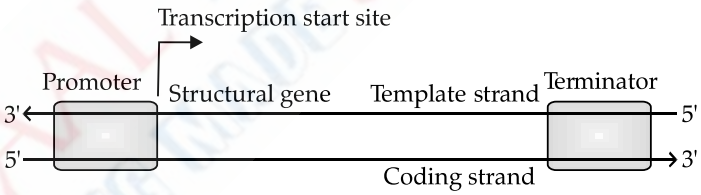

Fig 6.9 : Schematic structure of a transcription unit

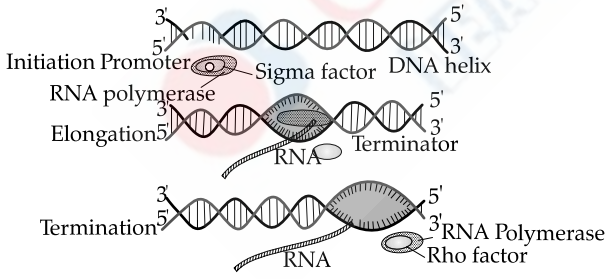

Fig 6.10 : Process of Transcription in Bacteria

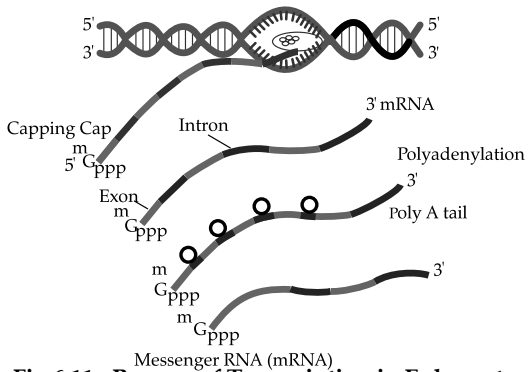

Fig 6.11 : Process of Transcription in Eukaryotes

TOPIC-2
Genetic Code, Translation, Lac Operon, Human Genome Project and DNA Fingerprinting

Revision Notes

- **Genetic Code** : It is the sequence of nucleotides in mRNA that contains information for protein synthesis (translation).

- 20 amino acids are involved in translation.
  - **George Gamow :** Suggested that for coding 20 amino acids, the code should be made up of 3 consecutive nucleotides.
  - **Har Gobind Khorana :** Developed the chemical method in synthesizing RNA molecules with defined combinations of bases (homopolymers and copolymers).
  - **Marshall Nirenberg :** Developed a cell-free system for protein synthesis.
  - *Severo Ochoa* (polynucleotide phosphorylase) enzyme is used to polymerize RNA with defined sequences in a template-independent manner.
- **Salient Features of Genetic Code**
  - The genetic code is a triplet code (three-letter code) where three adjacent nitrogen bases code for a single amino acid.
  - 61 codons code for amino acids. 3 codons (UAA, UAG and UGA) do not code for any amino acids. They function as stop codons (Termination codons or non-sense codons).
  - Genetic code is universal *e.g.* From bacteria to human UUU codes for Phenylalanine. Some exceptions are found in mitochondrial codons and in some protozoans.
  - No punctuations between adjacent codons (comma less code). The codon is read in mRNA in a continuous fashion.
  - Genetic code is non-overlapping.
  - A single amino acid is represented by many codons (except AUG for methionine and UGG for tryptophan). Such codons are called degenerate codons.
  - Genetic code is unambiguous and specific. *i.e.* one codon specifies for only one amino acid.
  - The codon is read in the 5' $\rightarrow$ 3' direction.
  - AUG has dual functions. It codes for Methionine (met) and also acts as an initiator codon. In eukaryotes, *methionine* is the first amino acid and *formyl methionine* is the first amino acid in prokaryotes.
- **Mutations and Genetic Code**
  - The relationships between genes and DNA are best understood by mutation studies.
  - Effects of large deletions and rearrangements in a segment of DNA may result in loss or gain of a gene and so a function.
  - A classical example of point mutation is a change of single base pair in the gene for beta globin chain of haemoglobin that results in the change of amino acid residue glutamate to valine. It results into a diseased condition called sickle cell anaemia.
  - Insertion or deletion of one or two bases changes the reading frame from the point of insertion or deletion.
  - When there is shifting of the reading frame due to insertion or deletion of the nucleotide, such mutation is known as frameshift mutation.
  - This forms the genetic basis of proof that the codon is a triplet and is read in a continuous manner.
- **The Adaptor Molecule – tRNA**
  - The tRNA is a molecule that has about 60% of its part double-stranded and the rest remains single stranded which has unpaired bases.
  - The tRNA has
    - **(a)** An anticodon (NODOC) loop that has bases complementary to the CODON with which it gets attached in mRNA.
    - **(b)** An amino acid acceptor end to which amino acid binds. This end or site lies at the 3' end & CCA–OH group. The 5' end bears G.
    - **(c) T Ψ C loop :** This is the site for attaching with the ribosome. This has some unusual bases like Ψ (pseudouridine) and ribothymidine.
    - **(d) DHU-Loop :** It is the binding site for the enzyme aminoacyl synthetase. It is the largest loop and has Dihydrouridine.
    - **(e) Extra arm :** It is a variable side arm lying between T Ψ C and anticodon loop.
  - tRNA is called an adaptor molecule because it picks up amino acids from the cytoplasm and transfers them to ribosomes during protein synthesis.
  - For initiation, there is another tRNA called initiator tRNA.
  - There are no tRNAs for stop codons.
  - 2-D structure of tRNA looks like a clover-leaf according to Robert Holly (1965). The 3-D structure looks like inverted 'L' according to Klug (1974).
- **Translation – Protein Synthesis**

  It takes place in ribosomes. It includes 4 steps :

  1. **Charging of tRNA (aminoacylation of tRNA)**
     - Formation of a peptide bond requires energy obtained from ATP.
     - For this, amino acids are activated (amino acid + ATP) and linked to their cognate tRNA in the presence of *aminoacyl tRNA synthetase*. So, the tRNA becomes charged.

2. **Initiation**
   - It begins at the 5′-end of mRNA in the presence of an *initiation factor*.
   - The mRNA binds to the small subunit of the ribosome. Now the large subunit binds to the small subunit to complete the initiation complex.
   - Large subunit has 2 binding sites for tRNA- aminoacyl tRNA binding site (A site) and peptidyl site (P site).
   - Initiation codon for methionine is AUG. So, methionyl tRNA complex would have UAC at the anticodon site.
3. **Elongation**
   - At the P-site the first codon of mRNA binds with anticodon of methionyl tRNA complex.
   - Another aminoacyl tRNA complex with an appropriate amino acid enters the ribosome and attaches to A site.
   - Its anticodon binds to the second codon on the mRNA and a peptide bond is formed between first and second amino acids in presence of an enzyme, *peptidyl transferase*.
   - The uncharged tRNA moves from the P site to the E site and the peptidyl-tRNA moves to the P site. This is called a translocation.
   - Then 3rd codon comes into A site and a suitable tRNA with 3rd amino acid binds at the A site. This process is repeated.
   - A group of ribosomes associated with a single mRNA for translation is called a polyribosome (polysomes).
   - A ribozyme is a ribonucleic acid (RNA) enzyme that catalyses a chemical reaction. The ribozyme catalyses specific reactions in a similar way to that of *protein synthesis*. Also called catalytic RNA, ribozyme are found in ribosome where they join amino acids together to form *protein* chains.
4. **Termination**
   - When aminoacyl tRNA reaches the termination codon like UAA, UAG & UGA, the termination of translation occurs. The polypeptide and tRNA are released from the ribosomes.
   - The ribosome dissociates into large and small subunits at the end of protein synthesis.

   An mRNA has additional sequences that are not translated (untranslated regions or UTR). UTRs are present at both 5′-end (before start codon) and 3′-end (after stop codon). They are required for an efficient translation process.

➢ **Regulation of Gene Expression**

Gene expression results in the formation of a polypeptide. In eukaryotes, the regulation includes the following levels :

- Transcriptional level (formation of primary transcript).
- Processing level (regulation of splicing).
- Transport of mRNA from the nucleus to the cytoplasm.
- Translational level.

➢ **Importance of regulation of gene expression:**

- Gene regulation is the process to switch off or switch on the genes as per the requirement of the organism.
- Gene regulation is required so that there is no waste of energy in expressing the genes not required at the time.
- However, there are housekeeping genes that are always expressed in the cell.

The metabolic, physiological and environmental conditions regulate the expression of genes. *e.g.*,

- In *E. coli*, the enzyme *beta-galactosidase* hydrolyses lactose into galactose and glucose. In the absence of lactose, the synthesis of beta-galactosidase stops.
- The development and differentiation of an embryo into an adult the result of the regulation of several set of genes.

➢ **Operon Concept :** This is a regulatory system that is observed in bacteria.

- "Each metabolic reaction is controlled by a set of genes".
- All the genes regulating a metabolic reaction constitute an *Operon e.g., lac* operon, *trp* operon, *ara* operon, *his* operon, *val* operon etc.
- When a substrate is added to growth medium of bacteria, a set of genes is switched on to metabolize it. This is called induction.
- When a metabolite (product) is added, the genes to produce it are turned off. This is called repression.

➢ **The Lac Operon**

- **Lac operon in *E. coli* :** The operon controlling lactose metabolism. It consists of a regulator gene, 3-structural genes, an operator gene, promoter gene, a repressor and an inducer.
  - **(a) A regulatory or inhibitor (i) gene :** Codes for the repressor.
  - **(b) 3 structural genes :**
    - **(i) z gene :** Codes for β-*galactosidase* (hydrolyze lactose to galactose and glucose).
    - **(ii) y gene :** Codes for *permease* (increase permeability of the cell to lactose).
    - **(iii) a gene :** Codes for a *transacetylase*.
- The genes present in the operon function together in the same or related metabolic pathway. There is an **operator** region for each operon.
- If there is no lactose (inducer), lac operon remains switched off. In the absence of inducer, repressor gene is active. The regulator gene synthesizes mRNA to produce the **repressor protein**, this protein binds to the operator genes and blocks RNA polymerase movement. So the structural genes are not expressed.
- In the absence of glucose, If lactose is provided in the growth medium, the lactose is transported into the *E. coli* cells by the action of permease. Lactose (inducer) binds with repressor protein.
- So, repressor protein cannot bind to **operator gene**. The operator gene becomes free and induces the RNA polymerase to bind with **promoter gene** then transcription starts. Regulation of lac operon by repressor is called negative regulation.

➢ **Human Genome Project (HGP)**

- The entire DNA in the haploid set of chromosome of an organism is called a Genome.
- In human genome, DNA is packed in 23 chromosomes.
- Human Genome Project (1990-2003) is the first effort in identifying the sequence of nucleotides and mapping of all the genes in the human genome.
- Human genome contains about 3×10^9 bp.

➢ **Goals of HGP**

(a) To identify all the estimated genes in human DNA.
(b) To determine the sequences of the 3 billion chemical base pairs that make up human DNA.
(c) To store this information in databases.
(d) To improve tools for data analysis.
(e) To transfer related technologies developed during the project of society to other sectors of society.
(f) To address the ethical, legal and social issues (ELSI) that may arise from the project.

➢ **HGP was Closely Associated with Bioinformatics**

The application of computer science and information technology to the field of biology and medicine helps in analyzing DNA sequence data.

➢ **Methodologies of HGP**

There are two major approaches namely, ESTs and sequence annotation.

- **Expressed Sequence Tags (ESTs) :** Focused on identifying all the genes that are expressed as RNA and sequencing the same.
- **Sequence annotation :** Sequencing whole set of the genome containing all the coding & non-coding regions and later assigning functions to different regions.

➢ **Procedure :**

Isolate total DNA from a cell → Convert into random fragments of smaller size → Clone in suitable host (*e.g.* BAC – bacterial artificial chromosomes & YAC – yeast artificial chromosomes) for amplification through PCR (polymerase chain reaction) → Fragments are sequenced using Automated DNA sequencers (using Frederick Sanger method) → Sequences are arranged based on the overlapping regions → Alignment of sequences using computer-based programs → Genetic and physical maps on the genome were generated using the information on polymorphism of restriction endonuclease recognition sites and some repetitive DNA sequences (micro-satellites).

➢ **Salient Features of Human Genome**

(a) Human genome contains 3164.7 million nucleotide bases pairs.
(b) Total number of genes = about 25,000.

(c) Average gene consists of 3000 bases, but sizes vary. The largest known human gene (dystrophin on X-chromosome) contains 2.4 million bases.

(d) 99.9% of nucleotide bases are identical in all people. It is 0.1% which makes each of us unique.

(e) Functions of over 50% of discovered genes are unknown.

(f) Chromosome I has the most genes (2968) and Y has the fewest (231).

(g) Less than 2% of the genome codes for proteins.

(h) Repeated sequences make up a very large portion of the human genome. Repetitive sequences are stretches of DNA sequences that are repeated many times. They have no direct coding functions but they shed light on chromosome structure, dynamics and evolution.

(i) About 1.4 million locations where single-base DNA differences (SNPs- Single nucleotide polymorphism or 'snips') occur in humans.

➢ **DNA Fingerprinting (DNA profiling)**

- It is the technique to compare the DNA fragments of two individuals.
- Developed by **Alec Jeffreys (1985)**. He is considered as the father of DNA fingerprinting. Lalji Singh is the Father of Indian DNA fingerprinting.

➢ **Basis of DNA Fingerprinting**

- DNA carries some non-coding sequences called repetitive sequence [Variable Number Tandem Repeats (VNTR)].
- Number of repeats is specific. It varies from person to person and is specific to a person.
- The size of VNTR varies from 0.1 to 20 kb.
- Repetitive DNA is separated from bulk genomic DNA as different peaks during density gradient centrifugation.
- The bulk DNA forms a major peak and the other small peaks are called satellite DNA.
- Satellite DNA is classified into many categories (micro-satellites, mini-satellites etc) based on the base composition (A-T rich or G-C rich), length of segment and number of repetitive units.
- An inheritable mutation observed in a population at high frequency is called DNA polymorphism (variation at genetic level).
- Polymorphism is higher in non-coding DNA sequence. This is because mutations in these sequences may not have any immediate effect on an individual's reproductive ability.
- These mutations accumulate generation after generation and cause polymorphism. For evolution & speciation, polymorphisms play an important role.

➢ **Steps of DNA Fingerprinting (Southern Blotting Technique)**

(a) Isolate DNA (from any cells like blood stains, semen stains or hair roots).

(b) Make copies (amplification) of DNA by Polymerase Chain Reaction (PCR) if the amount of isolated DNA is small.

(c) Digest DNA by restriction endonucleases.

(d) Separate DNA fragments by gel electrophoresis over agarose polymer gel.

(e) Treat with alkali solution (NaOH) to denature DNA bonds so as to split them into single-stranded DNAs in the gel.

(f) Transfer (blotting) single-stranded DNA fragments to synthetic membranes such as nitrocellulose or nylon, and then baked in a vacuum oven at 80°C for 3-5 hours (to fix the DNA fragment on the membrane).

(g) Nitrocellulose filter membrane is placed in a solution containing a radioactive labelled single-stranded DNA probe. The DNA probes are small radioactive synthetic DNA segments of known sequences of nitrogen bases. These DNA probe binds with the complementary sequences of the DNA fragment on the membrane to form a hybridized DNA.

(h) The filter paper is washed to remove unbound probe.

(i) The hybridized DNA is photographed on to an X-ray film by autoradiography. The image (in the form of dark & light bands) obtained is called a DNA fingerprint. This gives the characteristic pattern of an individual's DNA.

➢ **Applications of DNA Fingerprinting are :**

- Forensic tool to solve paternity, rape, murder, etc.
- For the diagnosis of genetic diseases.
- To determine the phylogenetic status of animals.

IMPORTANT DIAGRAMS :

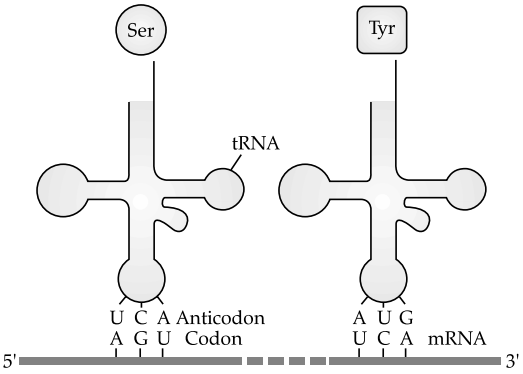

Fig 6.12: tRNA- the adapter molecule

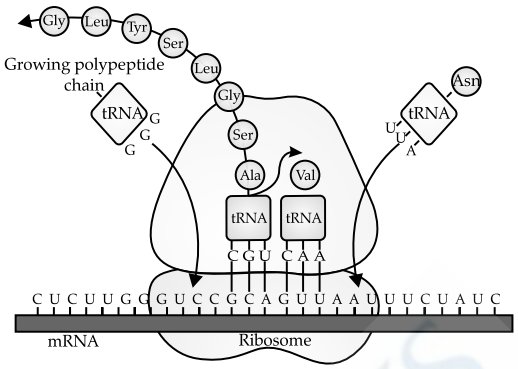

Fig 6.13: Translation

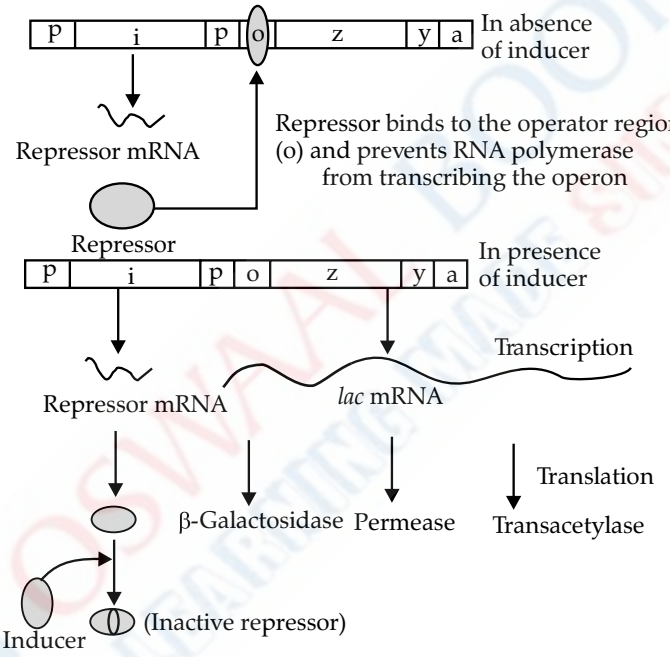

Fig 6.14: The *lac* Operon

Know the Terms

- **Anticodon :** A sequence of three nitrogeneous bases on tRNA which is complementary to the codon on mRNA.
- **BAC :** Bacterial Artificial Chromosome.
- **Capping :** Addition of methyl guanosine triphosphate to the 5′ end of hnRNA.
- **DNA Polymorphism :** The variations at genetic level, where an inheritable mutation is observed.
- **Euchromatin :** The region of chromatin which is loosely packed and genetically active.
- **Exons :** The regions of a gene which become part of mRNA and code for different regions of proteins.
- **Heterochromatin :** The chromatin that is more densely packed, stains dark and is genetically inactive.
- **HGP :** Human Genome Project.
- **hnRNA :** Heterogeneous nuclear RNA. It is precursor of mRNA.
- **Introns :** The regions of a gene which are removed during the processing of mRNA.
- **IRGSP :** International Rice Genome sequencing Project.
- **Nucleosome :** The structure formed when negatively charged DNA is wrapped around positively charged histone octamer.

- **Operon :** A group of genes which control a metabolic pathway.
- **Replication fork :** The Y shaped structure formed when double stranded DNA is unwound upto a point during its replication.
- **Satellite DNA :** The repetitive DNA sequences which form a large portion of genome and have high degree of polymorphism but do not code for any proteins.
- **SNPs :** Single Nucleotide Polymorphism.
- **Splicing :** The process in eukaryotic genes in which introns are removed and the exons are joined together to form mRNA.
- **Transcription :** The process of copying genetic information from one strand of DNA into RNA.
- **Transformation :** The phenomenon by which the DNA isolated from one type of a cell, when introduced into another type is able to express some of the properties of the former into the latter.
- **Translation :** The process of polymerisation of amino-acids to form a polypeptide as dictated by mRNA.
- **VNTR :** Variable Number of Tandem Repeats.
- **YAC :** Yeast Artificial Chromosome.

❑❑