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M.Sc. (Zoology)

II - Semester

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DEVELOPMENTAL BIOLOGY AND EVOLUTION

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SYLLABI-BOOK MAPPING TABLE

Developmental Biology and Evolution

Syllabi	Mapping in Book
BLOCK - I: GAMETOGENESIS & FERTILIZATION **UNIT 1:** General introduction to Developmental Biology, Spermatogenesis, Physiological maturation of sperm, Sperm structure and physiology. **Unit II:** Oogenesis, Egg - size, shape, Egg membranes and organization of egg - yolk, pigments, egg cortex. **Unit III:** Maturation of egg, Polarity and Symmetry, Classification of eggs. **Unit IV:** Fertilization : Types and Mechanism, Monospermy and Polyspermy. Activation of egg and Egg metabolism.	**Unit 1:** Developmental Biology: General Introduction **(Pages 1-23);** **Unit 2:** Oogenesis, Egg: Membranes and Structure **(Pages 24-40);** **Unit 3:** Eggs: Maturation and Classification **(Pages 41-56);** **Unit 4:** Mechanism of Fertilization **(Pages 57-73)**
BLOCK – II: CLEAVAGE AND GASTRULATION **Unit V:** Types of cleavage, Factors affecting cleavage, Chemodifferentiation. **Unit VI:** Blastulation, Types of blastula – Gastrulation in frog and chick, Mechanism of morphogenetic movement. **Unit VII:** Metabolic and molecular changes during gastrulation; Cell motility and Differential cell affinity; Fate maps construction.	**Unit 5:** Cleavage and Chemodifferentiation **(Pages 74-88)** **Unit 6:** Blastulation and Gastrulation in Frog and Chick **(Pages 89-114)** **Unit 7:** Metabolic and Molecular Changes During Gastrulation **(Pages 115-137)**
BLOCK – III: ORGANOGENESIS AND ASSISTED REPRODUCTIVE TECHNOLOGY **Unit VIII:** Development of eye, brain and heart in chick. Formation of muscle and neural crest. Embryonic induction, concept of organizer. **Unit IX:** Foetal membranes in chick, placenta in mammals. Origin of gene theory, Nuclear transplantation, Differential gene activation, Factors involved in teratogenesis. **Unit X:** Concept of Assisted Reproductive Technology (ART) – Monitoring of ovulation phase, Super-ovulation and Cryopreservation. **Unit XI:** Sperm banking, Artificial insemination, IVF, Embryo transfer and Test tube babies, Gene knock out and knock in.	**Unit 8:** Embryonic Development Stages of Chick **(Pages 138-163)** **Unit 9:** Foetal Membranes, Placenta and Gene Theory **(Pages 164-190)** **Unit 10:** Assisted Reproductive Technology **(Pages 191-216)** **Unit 11:** Sperm and Artificial Insemination **(Pages 217-248)**

BLOCK – IV: EVOLUTION

Unit XII: Lamarckism, Neolamarckism, Darwinism, Neodarwinism, Theory of natural selection, Genetic and non-genetic variations and Evolution of races to species.

Unit XIII: Evidences for evolution (Anatomical, Embryological, Physiological and Biochemical).

Patterns of behavioural adaptations, Isolating mechanism and speciation.

Unit XIV: Evolution of gene families, Molecular drive, Assessment of molecular variation, human origin and migration, Phylogenetic tree at molecular level.

CONTENTS

INTRODUCTION

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Developmental biology is the study of the process by which animals and plants grow and develop. Fundamentally, the developmental biology encompasses the biology of embryonic development, regeneration, asexual reproduction, metamorphosis, and the growth and differentiation of stem cells. The main processes involved in the embryonic development of animals include the regional specification, morphogenesis, cell differentiation, growth, and the overall control of timing explored in evolutionary developmental biology.

Evolution is referred as change in the heritable characteristics of biological populations over successive generations. These characteristics are the expressions of genes that are passed on from parent to offspring during reproduction. Different characteristics tend to exist within any given population as a result of mutation, genetic recombination and other sources of genetic variation. Principally, the evolution occurs when evolutionary processes, such as natural selection and genetic drift act on this variation, resulting in certain characteristics becoming more common or rare within a population.

This book, *Developmental Biology and Evolution*, is divided into four blocks that are further divided into fourteen units which will help to understand the basic concepts of developmental biology and evolution of animals, gametogenesis and fertilization, spermatogenesis, physiological maturation of sperm, oogenesis, egg membranes and organization of egg (yolk, pigments, egg cortex), maturation of egg, polarity and symmetry, classification of eggs, fertilization - types and mechanism, monospermy and polyspermy, cleavage and gastrulation, chemodifferentiation, blastulation, gastrulation in frog and chick, mechanism of morphogenetic movement, metabolic and molecular changes during gastrulation, organogenesis and assisted reproductive technology, development of eye, brain and heart in chick, formation of muscle and neural crest, embryonic induction, concept of organizer, foetal membranes in chick, placenta in mammals, origin of gene theory, nuclear transplantation, differential gene activation, assisted reproductive technology, monitoring of ovulation phase, super-ovulation and cryopreservation, artificial insemination, IVF, embryo transfer and test tube babies, evolution (Lamarckism, Neo-Lamarckism, Darwinism, Neo-Darwinism) theory of natural selection, genetic and non-genetic variations and evolution of races to species, evidences for evolution (anatomical, embryological, physiological and biochemical), patterns of behavioural adaptations, isolating mechanism and speciation, evolution of gene families, molecular drive, human origin and migration.

The book follows the self-instruction mode or the SIM format wherein each unit begins with an 'Introduction' to the topic followed by an outline of the 'Objectives'. The content is presented in a simple, organized and comprehensive form interspersed with 'Check Your Progress' questions and answers for better understanding of the topics covered. A list of 'Key Words' along with a 'Summary' and a set of 'Self Assessment Questions and Exercises' is provided at the end of the each unit for effective recapitulation.

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BLOCK - I

GAMETOGENESIS AND FERTILIZATION

UNIT 1 DEVELOPMENTAL BIOLOGY: GENERAL INTRODUCTION

Structure

1.0 INTRODUCTION

Developmental biology is the field of science that explores the potential of an unorganized fertilized cell to form a definite adult animal. Continuity of life through mysterious embryonic development is an organized process that begins with the formation of microscopic structures called as gametes. Gametes are the cells produced by parent generation though the process of gametogenesis and are laden with hereditary information. Gamete formation is a complex process which involves a structured division of diploid cell (Germ cell) into haploid gamete. Gametes in case of male are called as sperms and egg in case of females. As the name suggests, the process of sperm formation is known as spermatogenesis and that of egg formation is called as oogenesis. Both these processes undergo a series of events under the influence of specific hormones (different in male and female) to form gametes. These gametes from opposite sex fuse with each other to blend the genetic information through a complex process called as fertilization.

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This give rise to the formation of a diploid structure called as Zygote, which is the first diploid cell of the body. The whole process is divided into two phases, i.e., Pre-zygotic and Post-zygotic. The sequence of diploid cell followed by haploid cell and the again diploid cell is the secret that maintains the chromosome number from generation to generation. Detailed description of all the events is given in the present chapter.

In this unit, you will study about the history, introduction and scope of developmental biology, gametogenesis with special reference to spermatogenesis, physiology of sperm maturation and the structure and physiology of sperm in detail.

1.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand the history, introduction and scope of developmental biology
- Explain the gametogenesis with special reference to spermatogenesis
- Discuss the physiology of sperm maturation
- Describe the structure and physiology of sperm

1.2 INTRODUCTION TO DEVELOPMENTAL BIOLOGY

From the day of conception of female till the death of an organism, all the dramatic changes encountered by an organism for its growth and development can be studied only under the field of biology known as Developmental biology. Answer to all the bizarre questions like how organisms develop? Why we are having only five fingers? How is the body formed with its head above the shoulder? Why is the heart placed at the left side of the body? Why can only female give birth to young ones; Why not males? Why human cannot regenerate limbs whereas frog does? So the Developmental biology is the field which can answer all the questions that comes in the researcher's mind as well as to that of a layman. The study of development has become essential for understanding all the other areas of biology. According to Franklin Mall, it is the most beautiful thing to study is the different changes in life from the microscopic changes of conception to the more apparent ones of maturity and old age.

Developmental biology deals with detailed study of an organism from the day of zygote formation till the death of an organism and can be used in curing various congenital abnormalities and disorders. Developmental biological studies also comprises the biology of regeneration, metamorphosis, asexual reproduction, growth and differentiation of stem cells, teratogenesis, etc.

Earlier developmental biology was considered as the synonym of the embryology however both the studies are different from each other on the basis of

their approach. On the one hand, embryology is a slow process of progressive changes by which a multicellular organism is formed during the course of development whereas developmental biology deals with the mechanisms of these progressive changes. Traditionally, embryology has been defined as the study of the animal development from phase of an organism, i.e., between fertilization and birth although developmental biology infer study of organism right from the zygote formation till the death of the organism as the development neither stops at the time of birth nor even at childhood. Replacement of skin cells, generation of new blood cells and regeneration of body parts, transformations from the larval stage to adult (metamorphosis); all are the evidences of the sustainable development. According to the Aristotle, first embryologist known to history he said the 'it is owing to wonder that people began to phiolosophisize and wonder remains the beginning of knowledge' (Refer Figure 1.1).

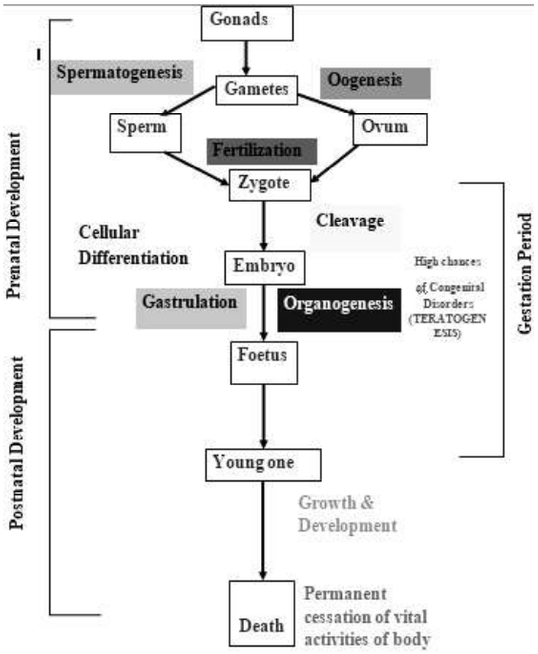

Fig. 1.1 *Outline of Scope of Developmental Biology*

1.2.1 History and Scope of Developmental Biology

The development of multicellular organisms from a single cell, the fertilized egg is a brilliant triumph of evolution. During embryonic development, the egg divides to give rise to many millions of cells, which forms structures as complex and varied as eyes, arms, heart, and brain. Much of the excitement in developmental biology today comes from our growing understanding of how genes direct these developmental processes.

The process of progressing from a single cell through the period of establishing organ primordial (the first 8 week of human development) is called the period of

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embryogenesis or organogenesis. The period from this onwards up to the birth is called the fetal period, a time when differentiation continues while the fetus grows and gain weight. The scientific approaches to study embryology have progressed over hundreds of years. The comparative and evolutionary studies help to understand the progression of developmental phenomena. The scientists by the application this technique compared the pattern of development among different groups to investigate either the developing offspring are either normal or with birth defects. The study of the embryological origins and cause for these birth defects was called teratology. In the 20th century, the field of experimental embryology blossomed. Numerous experiments were devised to trace cells during development to determine their cell lineages. The hundreds years of journey of developmental biology and contribution of many eminent scientists are summarized below in Table 1.1:

Table 1.1 Summary to show Eminent Scientists and their Contribution in Developmental Biology

Name of Scientists	**Contribution to Developmental biology**
Hippocrates (5th Century BC)	Scientific approach for development started.
Aristotle (384-322BC)	First defined the epigenesist and preformation.
Fabricius (1600-1621)	Published first book on Embryology.
William Harvey (1578-1657)	Noticed blastoderm of chick embryo, Quoted '*Omne vivum ex ovo'*, i.e., all life forms originated from a single egg.
Regnier de Graaf (1641-1673)	Described the mature ovarian follicle in 1672.
Leeuwenhoek (1632-1723)	Discovered spermatozoa in human semen with self designed microscope.
Charles Bonnet (1720-1793)	First to recognize the act of natural parthenogenesis.
Christian Pander (1794-1865)	Described the origin of three germ layers in chick embryo. Discovered Tissue interaction (Induction).
Karl Ernst Von Baer (1792-1876)	Discovered the mammalian ovum, and notochord in 1827.
Theodore Schleiden (1804-1881)	Established the cellular nature of ovum.
George Newport (1803-1857)	Described the entry of sperm into frog's egg in 1854.
Schweigger-Seidel and St. George	Examined the cellular nature of sperm in 1865.
Oscar Hertwig (1849-1922)	Explained the union of male and female gamete nuclei during fertilization in sea urchin.
Van Beneden	Explained the behavior of chromosomes in fertilization in 1883.
Spallanzani	First to observe process of cleavage in frog's egg.
R. Hertwig and E. Manupas	Demonstrated the exchange of micronuclei during conjugation in *Paramecium* in 1889.
Oscar Hertwig	Explained the effect of yolk content in the egg affecting the cleavage and its types in 1897.
Jaeques Loeb (1809-1924)	Induced artificial parthenogenesis in sea urchin and frog eggs in 1900.
	Explained the concept of an organizer in 1921.
J. Holtfreter	Explained the concept of inducers in 1933.
G.G. Pincus	Artificially produced first mammalian (rabbit) parthenogenetic offspring in 1936.

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1.2.2 Developmental Model Organisms

An organism suitable for studying a specific trait, disease, or phenomenon, having short generation time, characterized genome or similarity to humans is known as model organism. Examples include a fly, fish, rodent or pig, whose biology is well known and accessible for laboratory studies. In recent years the utmost developmental study has focused on the use of a small number of model organisms emphasizing much conservation of developmental mechanisms across the animal kingdom. In early development, different vertebrate species use essentially the same inductive signals and the same genes encoding regional identity. Even invertebrates use similar signal pathways and genes although the organogenesis varies significantly. Each model organism has some particular experimental advantages which enable them to become popular among researchers.

In one sense they are 'models' for the whole animal kingdom, and in another sense they are 'models' for human development, which is difficult to study directly either due to ethical reasons or practical reasons or sometimes both. Model organisms have been most useful for elucidating the broad nature of developmental mechanisms. There are different model organisms in different groups of organisms such as: plants (plants: *Arabidopsis thaliana*; invertebrates: *Drosophila*, *Caenorhabditis elegans*, sea urchins; and vertebrates: ascidians, *Xenopus*, *Danio, Gallus, Mus*). For studies of regeneration axolotl larva of *Ambystoma mexicanum* and planarian worms are used. The organoids have also been demonstrated as an efficient model for development.

1.2.3 Applications of Developmental Biology

There are wide applications of developmental biology of which some are as follow:

- Growing field of stem cell research ranging from basic research to clinical applications by using embryonic stem cells, mesenchymal stem cells or induced stem cells.
- Embryos of various species like mice, chicken and zebra fish are often used to investigate genetic, physiological or pharmacological influences on organogenesis or disease progression.
- Embryonic models are widely used in treatment of cardiovascular research.
- Field of developmental biology helps in the evolutionary studies and help in tracing the occurrence of vestigial structures.
- Study of various developmental processes helps in revealing mysterious fundamental life processes such as regeneration, metamorphosis, teratogenesis, etc.
- Detailed study of embryonic phases of developing embryo could be helpful in treating various congenital abnormalities caused due to various extrinsic factors often called as teratogens.

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- By understanding the detailed phenomenon involved in the various developmental processes, it will be possible to develop new drugs or therapies for the prophylaxis of various disorders.
- According to the US National research Council, over half of the initial pregnancies are affected by the developmental birth defects, in which 70% are the neonatal deaths. So the study of developmental biology plays a crucial role in reducing the number of neonatal deaths as it helps in studying the detailed mechanisms of exposure of embryo to the exogenous factors responsible for birth defects.

Check Your Progress

1. Name the first embryologist known to history?
2. Who discovered the spermatozoa?
3. Enlist some organisms used extensively in developmental biology.

1.3 GAMETOGENESIS

For successful reproduction the utmost need is the production of functional gametes by the organism. Gametes are haploid reproductive cells which carries haploid set (in case of humans n=23) of chromosomes and are formed from diploid gonads (Testes in case of males and ovaries in case of females). Gametes are produced by specialized cell division known as reductional division or meiosis. Although both spermatozoa and egg are morphologically distinct but both undergo similar stages of development. Gametes are the physical carriers of genetic information which transmit parental characters from one generation to next generation. In case of males the gamete is sperm where as in case of females the gamete is known as Egg.

Collectively the process of gamete formation is called as gametogenesis and is of two types:

- Spermatogenesis
- Oogenesis

1.3.1 Spermatogenesis

Spermatogenesis is the developmental process from germ cell that ends at formation of haploid spermatid, which begins at the puberty and occurs in the seminiferous tubules of the testis. Once the process of spermatogenesis starts, it is a continuous process and occurs throughout the life of a male. The process of spermatogenesis is divided into three major phases: i. Proliferative phase or Multiplicative phase, ii. Meiotic phase, and iii. Post Meiotic phase (Maturation phase) (Refer Figure 1.2 and 1.3).

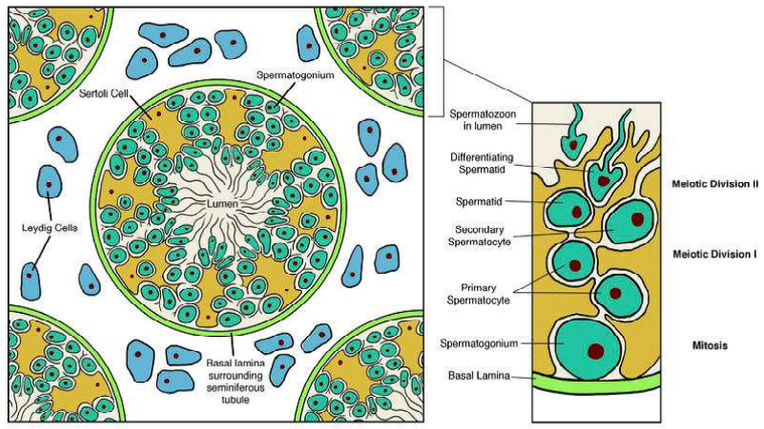

Fig.1.2 *Seminiferous Tubule, Cross section of a Testis and Seminiferous Tubules*

Proliferative Phase

It involves the rapid multiplications of primary germ cells which begin with the arrival of Primordial Germ Cell (PGCs) (also known as Gonocytes) at genital ridge of the male embryo. These Gonocytes get incorporated into the sex cord which becomes seminiferous tubules with the course of development. The Gonocytes gets differentiated into the numerous stem cells known as type A_1 spermatogonia (Spermatogonia are the diploid cells with chromosome number 46 in case of human beings). These are true stem cells having the ability to reinitiate the process of spermatogenesis when transferred to mice whose sperm production is obliterated through injection of toxic chemicals. Spermatogonia resides in the stem cell niches at the junction of Sertoli cells (the epithelium of seminiferous tubules), the interstitial cells (Leydig cells) and the testicular blood vessels. There are adhesion molecules that join the Spermatogonia directly to the Sertoli cells which provide nourishment to them throughout the process of development. This mitotic proliferation of stem cells amplifies the small population of Gonocytes into a population of type A Spermatogonia which can generate more than 1000 spermatids per second in adult human male.

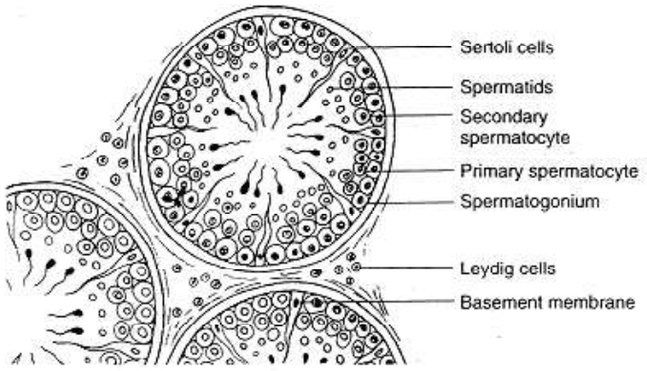

Fig.1.3 *T.S. of Testis to show Different Stages of Spermatogenesis*

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Meiotic Phase

The undifferentiated type A Spermatogonia are the sperm stem cells and that generate another type A Spermatogonia and type A_1 spermatogonium with the onset of puberty. Type A_1 spermatogonia have high level of Stra8 transcription factor and are committed to a meiotic pathway. They undergo five mitotic divisions but keep cytoplasmic connection intact between themselves. These cells form a syncitum in which each cell communicates with others via cytoplasmic bridges about 1 micrometer in diameter. There are chains of 2-32 linked A_1 Spermatogonia in human males. These cells divide to produce typeB Spermatogonia which are the precursors of the spermatocytes and are the last cells of the line that undergo mitosis. They divide once to generate Primary spermatocytes that enter into meiosis (Refer Figure 1.4).

The meiotic phase of spermatogenesis is regulated by numerous factors such as synthesis of BMPs by the Spermatogonia and synthesis of retinoic acid by the Sertoli cells during puberty. High concenteration of the BMP8b is needed for the differentiation of the germ cells. Studies have showed that mice lacks BMP8b factor and thus is not able to induce spermtogenesis at the age of puberty.

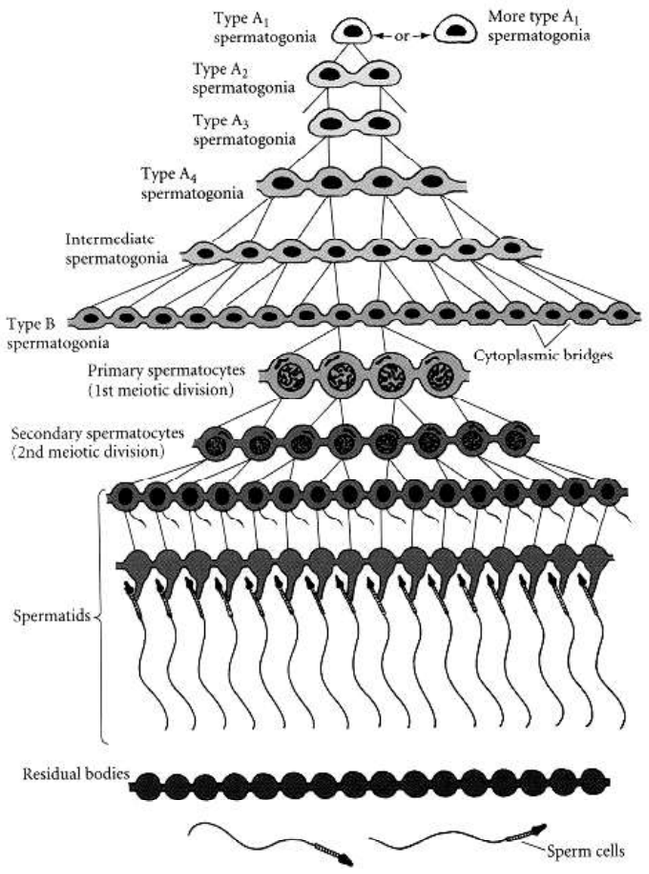

Fig.1.4 *Typical Spermatogenesis (Spermatocytogenesis and Spermiogenesis) in Vertebrates*

Maturation Phase

In maturation phase primary spermatocytes undergoes the first meiotic division and produce secondary spermatocytes (chromosome number 23). During the first meiotic division homologous chromosomes and their non-sister chromatids undergo the process of crossing over which leads to introduction of variations in the secondary spermatocyte. Meanwhile, the secondary oocyte undergo the second meiotic division and produce haploid cells called as spermatids that remain connected to one another through their cytoplasmic bridges which get separated from each other during the process of spermeogenesis. The spermaids are having haploid nuclei but are functionally diploid. During the division from type A1 spermatogonia to spermatids the cells move farther and farther away from the basal lamina of the seminiferous tubules into the lumen of the seminiferous tubules. Thus each type of cell can be found in a particular layer of the tubule. The spermatids are located at the border of the lumen where they lose their cytoplasmic connections and differentiate into spermatozoa. In human the progression from Spermatogonial stem cells to mature spermatozoa take 65 days (Refer Figure 1.5).

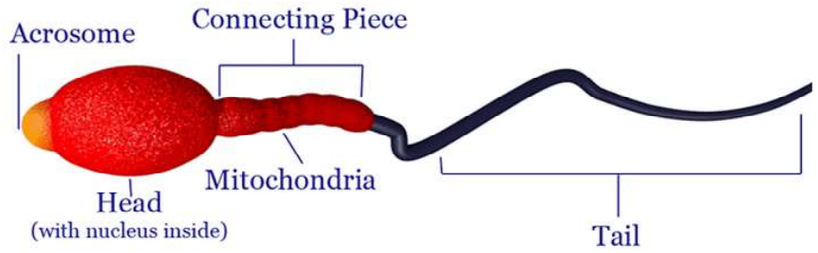

Fig. 1.5 *Mature Sperm Cell*

The transition between Spermatogonia and spermatocytes is mediated by the factors release by the Sertoli cells as follows:

- Glial cell lines- Derived Neurotrophic Factor (GDNF)
- Stem Cell Factor (SCF)

GDNF levels determine whether the dividing Spermatogonia last as the Spermatogonia or enter the meiotic phase to become spermatocytes. Low level of GDNF is required for the differentiation of Spermatogonia whereas high level of GDNF is needed for the self renewal of stem cells. SCF promotes the transition to spermatogenesis. Both GDNF & SCF are upregulated by FSH, so these two factors serves as link between Sertoli cells and the endocrine system which provide the mechanism for FSH to increase the production of sperms.

Post-Testicular Sperm Maturation

Studies have found that 'major maturation-associated' sperm membrane antigen (glycopeptides) appears on the surface of rat spermatozoa during post-testicular sperm maturation in the distal epididymis of rats. It has been found through in-situ

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transcript hybridization, molecular analyses of genomic DNA fragments and immunohistochemical staining that homologous counterparts are present in other mammals, including humans. In case of humans, this homologue is an abundant epididymal gene product that has been identified as lymphocyte surface antigen CD52. These Glycosylphosphatidyl Inositol (GPI)-anchored glycopeptides (absent in testis) are located within the epididymal epithelium that are transferred to sperm cell from epididymal through GPI-anchor; remains intact. Human TRPM 2/ clusterin, is another gene associated with sperm maturation.

Hormonal Control of Spermatogenesis

Three hormones produced by male gonads directly or indirectly control the process of spermatogenesis. They are- testosterone, estradiol and inhibin. Testosterone is secreted by leydig cells which are present adjacent to seminiferous tubules. Estradiol and inhibin are secreted by sertoli cells or interstitial cells which are present inside the seminiferous tubules. The initiation of the process of spermatogenesis greatly depends on the activity of hypothalamus and adenohypohysis (Refer Figure 1.6).

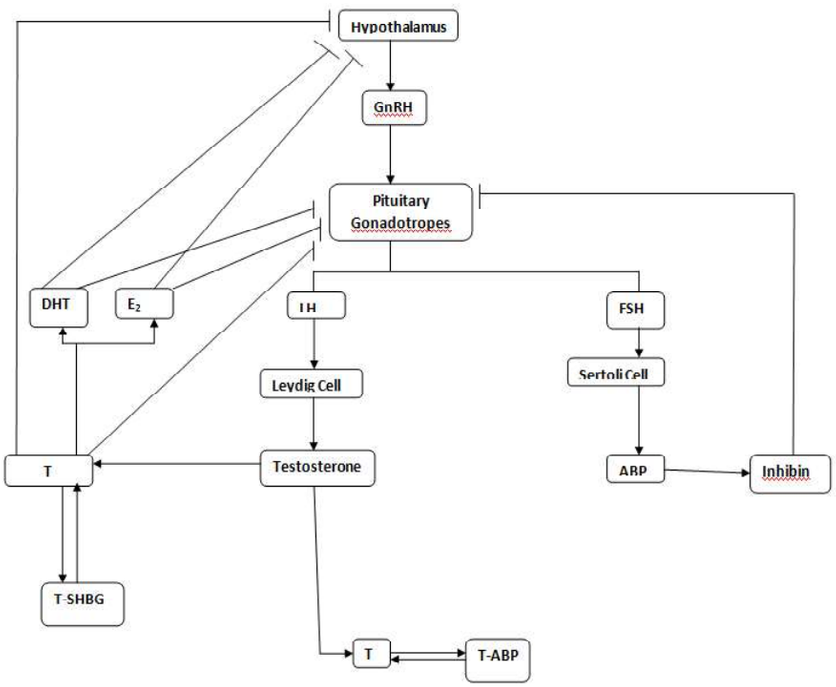

Fig. 1.6 *Diagrammatic Representation of Hormonal control of Spermatogenesis.*

Deficiency of Fibroblast Growth Factor 2 (FGF-2) leads to abnormal spermatogenesis and altered sperm physiology. Earlier it was revealed that the presence of Fibroblast Growth Factor 2 (FGF-2) and its receptors (FGFRs) in human testis and sperms are involved in spermatogenesis and in motility regulations. Some studies were carried out to analyze the role of FGF-2 in the maintenance of sperm physiology using FGF-2 Knock Out (KO) mice experiment which showed

that in Wild-Type (WT) animals, FGF-2 are expressed in germ cells of the seminiferous epithelium, in epithelial cells of the epididymis and in the flagellum and acrosomal region of epididymal sperm. This suggest that FGF-2 exerts a role in mammalian spermatogenesis and the lack of FGF-2 leads to deregulated sperm production as well as altered sperm morphology and function. FGF-2-deficient mice constitute a model for the study of the complex mechanisms underlying mammalian spermatogenesis.

Factors Affecting Spermatogenesis

Following are the factors that affect Spermatogenesis:

- **Temperature:** Normal body temperature or above it, sensitize the seminiferous epithelium. So the optimal temperature (2 degree below body temperature) is maintained by the scrotum.
- **Scrotal Reflex:** Cremasteric muscles and Dartos smooth muscles in scrotum, position it towards or away from the heat of the body.
- **Others:** Deficiency of vitamin E, B and A; infectious diseases; X-ray exposure, metals like lead and cadmium, dioxin, alcohol, pesticides and anabolic steroids, and DNA damage due to oxidative stress.

Check Your Progress

4. What are the factors responsible for regulation of meiotic phase of spermatogenesis?
5. Name the factor which deficiency leads to abnormal spermatogenesis and altered sperm physiology?

1.4 SPERM: PHYSIOLOGICAL MATURATION AND STRUCTURE

Sperm is the male reproductive cell and is derived from the Greek word *sperma* (meaning seed). In the types of sexual reproduction known as anisogamy and its subtype oogamy, there is a marked difference in the size of the gametes with the smaller one being termed the 'male' or sperm cell. A uniflagellar sperm cell that is motile is referred to as a **spermatozoon**, whereas a non-motile sperm cell is referred to as a **spermatium**. Sperm cells cannot divide and have a limited life span, but after fusion with egg cells during fertilization, a new organism begins developing, starting as a totipotent zygote. The human sperm cell is haploid, so that its 23 chromosomes can join the 23 chromosomes of the female egg to form a diploid cell. In mammals, sperm develops in the testicles, is stored in the epididymis, and released from the penis.

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The main sperm function is to reach the ovum and fuse with it to deliver two sub-cellular structures:

- Male pronucleus that contains the genetic material
- Centrioles that are structures that help organize the microtubule cytoskeleton

Related to sperm quality is sperm size, at least in some animals. For instance, the sperm of some species of fruit fly (*Drosophila*) are up to 5.8 cm long — about 20 times as long as the fly itself. Longer sperm cells are better than their shorter counterparts at displacing competitors from the female's seminal receptacle. The benefit to females is that only healthy males carry 'good' genes that can produce long sperm in sufficient quantities to outcompete their competitors (Refer Figure 1.7).

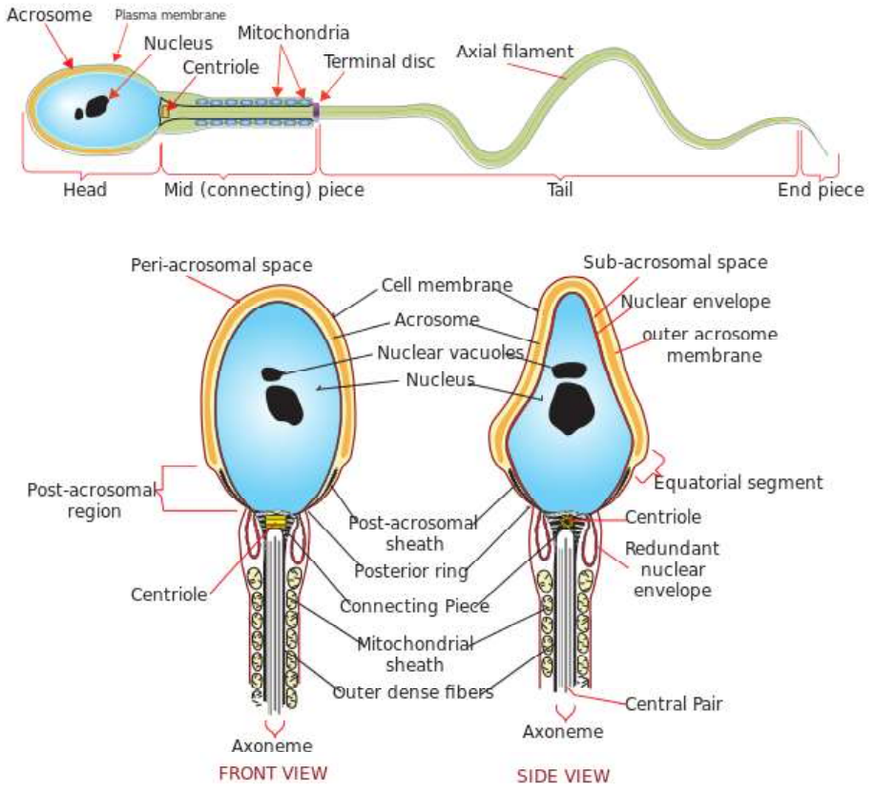

Fig. 1.7 *Human Sperm Cell*

Physiological Maturation of Sperm

Haploid spermatid is round, unflagellated cells that undergo transformation i.e. conversion of haploid, non-motile spermatid into motile elongated spermatozoa. Spermiogenesis is the process by which haploid round spermatid completes an extraordinary series of events to become streamlined motile spermatozoa. Spermiogenesis begins after spermatocytes complete two quick successive meiotic

reductive divisions to produce haploid round spermatids. Further no cell division occurs as the spermatids undergo complex cytodifferentiation, over a period of 2–3 weeks in mice and rats to form mature elongated spermatids that are ultimately released from the seminiferous epithelium through a process known as spermiation. Main significance of this process is to increase the sperm motility and to make sperm more potential so that it could easily penetrate the ovum and process of fertilization could takes place. During spermiogenesis the developing sperms keep their heads embedded in the sertoli cells so that they could easily draw the nourishment from them (Refer Figure 1.8). There are following processes involved during changes and maturation of sperms:

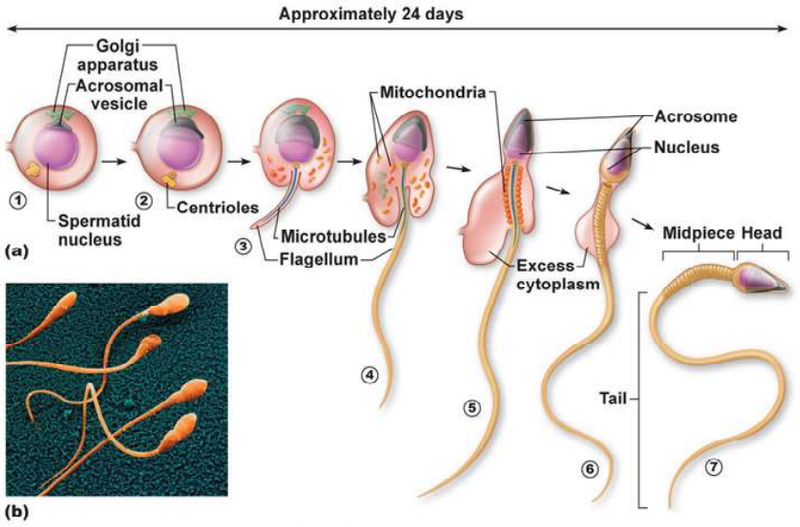

Fig. 1.8 *Representation of Changes during Spermiogenesis or Maturation of Sperms*

Changes in Nucleus: Initially the change that takes place in the process of spermatogesis is the progressive elongation and reduction in volume of the nucleus by losing water from the karyolymph. This step is essential, since it reduces the weight of the spermatozoa and enhances the sperm motility. Along with this condensation of DNA and reduction of RNA takes place. Chromatin fibers become closely packed and form uniformly dense mass. All these changes reduce the volume of nucleus to 0.5 %. In the compact chromatin, DNA is transcriptionally active therefore protein synthesis in spermiogenesis utilizes the stored mRNA molecule. This is an example of Post transcriptional regulation of gene expression. During all these changes that take place, nucleus attains a characteristic shape and forms the sperm head. Shape of the nucleus is determined by the pattern of DNA-protein interaction during the condensation.

Changes in Golgi Complex: Golgi apparatus of spermatid gathers anterior to the nucleus. Membrane bound pro-acrosomal vesicle develop in the Golgi apparatus. These proacrosomal granules accumulate into a single large Acrosomal Vesicle having dense Acrosomal granule in it. Acrosomal vesicle joins with nucleus

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and forms the future anterior end of the sperm. When the acrosomal vesicle attains its size, it assumes the final shape of mature acrosome.

Changes in Centerioles: Spermatid has two types of nucleus, during the process of spermiogenesis both the centrioles change their position and lie behind the nucleus. Out of these two centerioles, one enters into depression developed in the posterior part of the sperm and latter one is called as the proximal centriole. The remaining centeriole is known as distal centriole which will be present behind the proximal centeriole and its axis coinciding with the longitudinal axis of the spermatozoa.

There are following functions of centriole:

- Proximal centeriole enters inside the ovum along with sperm and plays a role in the spindle formation.
- Distal centeriole give rise to 9+2 axial filament of the flagellum and act as basal granule.
- In some Mammals Proximal and Distal Centeriole get disappears after organizing the connection between neck and middle piece.

Changes in Mitochondria: Mitochondria play an important role in the process of spermeiogenesis in the middle piece. It gets accumulated around the proximal part of the axial filament and distal centeriole .Gradually, these mitochondria loses their individuality and fuse together forming two densely packed bodies, one on the either side of axial filament (mammals only) and this sheath is known as Nebenkern (spiral arrangement of sheath is visible only in mammals). In other animals, mitochondria are joined to form massive clumps known as mitochondrial bodies (Refer Figure 1.9).

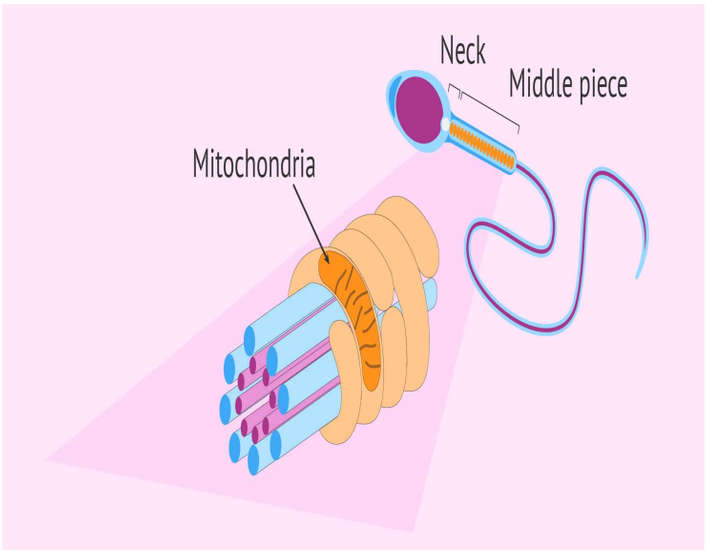

Fig. 1.9 *Mitochondria in a Sperm*

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Changes in Cytoplasm: Most of the cytoplasm in spermatid is lost and remaining cytoplasm forms a condensed layer in the peripheral region of the spermatid. This layer is known as Manchette band which surrounds the middle piece as well as the posterior part of sperm's head.

Changes in Plasma Membrane: Plasma membrane extends to surround the acrosome, nucleus and middle piece and tail of the sperm. Initially some proteins remain embedded over the surface of plasma membrane and acts as recognition or binding factors during the process of fertilization.

Hence, above mentioned are all the changes that take place during the conversion of spermatid into spermatozoa.

Metamorphic Changes: The different steps of spermiogenesis are distinguished by the morphological appearance of the developing acrosome and the change in the shape of the nucleus. During spermiogenesis, round spermatids having a spherical central nucleus, begin to assemble the acrosome and the axoneme structures required for the fertilization and motility respectively**.** Thus the major event occurring during spermiogenesis is the assembly of the sperm flagellum. The central component of the flagellum i.e. microtubule-based axoneme, is assembled soon after the completion of meiosis. As spermatids elongate, the accessory structures needed for flagella function (outer dense fibers, fibrous sheath, mitochondrial sheath) are assembled around the central axoneme. The final stage of spermiogenesis is known as spermiation and is the process by which the elongated spermatids undergo their final remodeling and get released from the seminiferous epithelium. Spermiation is a complex, multi-step process, which is particularly vulnerable to disruption. In case of mice, the last stage of sperm maturation is controlled by *Katnal1* gene. This is expressed in sertoli cells which supports and nurture sperm at the site of spermatogenesis. Dysfunction of *Katnal1* gene leads to male infertility.

1.4.1 Structure and Physiology of Sperms

Structure of Sperm

Sperm, also called spermatozoon (plural spermatozoa) is a male reproductive cell produced by most of the animals (Refer Figure 1.10 and 1.11). It is flattened and has a whip tail with the exception of nematode worms, diplopods (for example, millipedes), decapods (for example, crayfish) and mites. In higher vertebrates, especially mammals, testes produce the sperm which unites (fertilizes) with an egg (ovum) of the female to form a new offspring. The sperm cells have half of the usual number of the chromosomes (n=23). When sperm cell unites with ovum, (also has 23 chromosomes), the resulting cell called Zygote (n equal to 46 chromosomes) determine the offspring's characteristics . Moreover, the sperm cell also carry X or Y chromosome which determines the sex of the young one. Sperm is 60 μm long, actively motile and is divided into 3 main regions:

- Head
- Neck

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- Middle Piece
- Tail

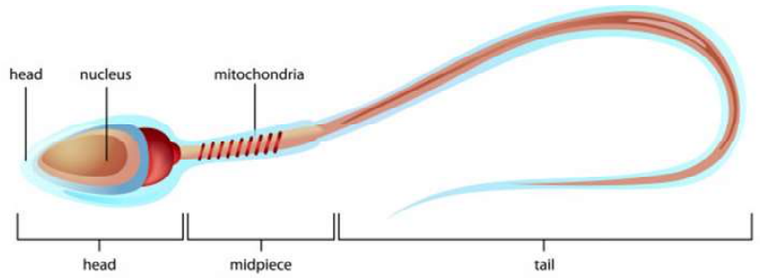

Fig. 1.10 *Sperm Cell Anatomy*

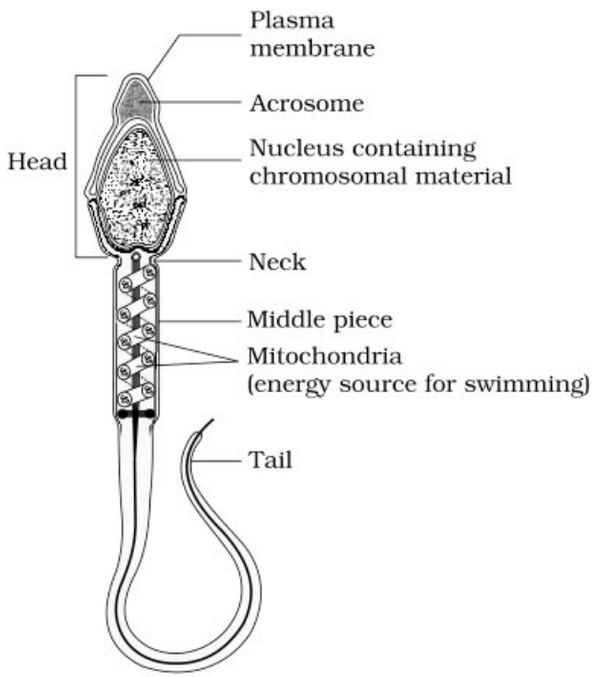

Fig.1.11 *A Typical Structure of Mature Sperm*

Head: The head of the sperm show variation in shape for different animal species. In humans it is flattened, almond-shaped, 4-5µm long and 2-3µm wide. The head portion is mainly a cell nucleus having genetic substances, known as chromosomes; that transmits specific characteristics of an individual, such as the colour of eyes, skin and hair. Each body cell of a healthy person has 46 chromosomes, which determine the individual's general physical makeup. Head of the sperm is covered by a cap called as acrosome that has enzymes which help sperm to enter an egg. An average ejaculation contains 300,000,000 to 400,000,000 sperm out of which only one enters the egg. Each egg and sperm produced shows slight difference in genetic information stored in the chromosomes

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which accounts for differences and similarities in between children of the same parents (Refer Figure 1.12).

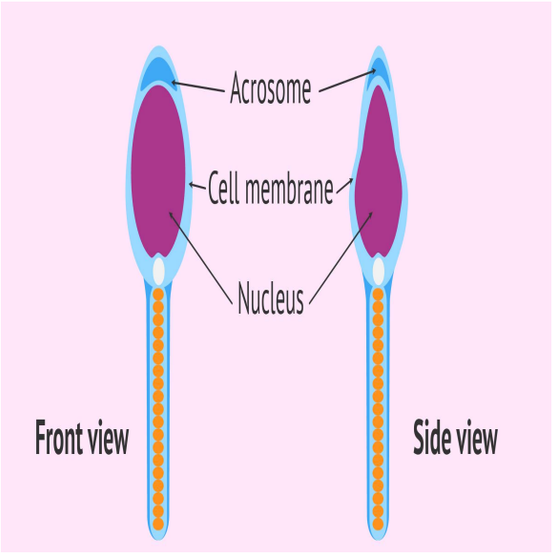

Fig. 1.12 *Head of Sperm*

Neck and Middle Piece: The neck is a constriction like structure of about 1µm length and is attached to basal plate, transverse oriented centriole, containing nine segmented column of fibrous materials and continues as outer dense fibers in tail. The middle piece is 5µm long; consist of axonema and dense fibers surrounded by mitochondria. The middle piece divisible in two parts: principal piece and end piece. The principal piece is about 4.5µm long fibrous sheath interconnected by regularly spaced circumferential hoops, while the end piece is about 0.5µm long having axonema surrounded by small amount of cytoplasm and plasma membrane.

Tail: It has three parts: a middle piece, principal piece, and end piece. A small middle portion of sperm contains mitochondria. The tail of sperm (sometimes called flagellum) is a slender, hair like bundle of filaments which connects with the head and middle portion. The tail is about 50µm long; having thickness of 1µm near the mitochondria which gradually diminishes to less than 0.5µm at the end of tail. The tail helps in sperm cell movement (whips and undulates) so that it can travel to the egg.

Physiology of Sperm

After sperm deposition in the reproductive tract of female, tail movement is suppressed until the sperm reaches relatively short distance of the egg. This facilitates the sperm with increased chance of reaching to the egg before it exhausts its energy supplies. The activation of tail movements is part of the process called as capacitation, in which the sperm undergoes a cascade of cellular changes to enables, its participation in the process of fertilization. Alkalinization of sperm cytoplasm is the elementary change that occurs during capacitation where increase

in pH levels takes place, mainly in the flagellum. This process takes place due to the rapid movement of protons out of cell via ion channels present on flagellum which leads to tail activation. Anandamide is the compound present in the female reproductive track (high concentration near the egg) plays an important role in opening the proton channels. When the sperm reaches the egg, the enzymes in the acrosome gets activated which all the sperm to penetrate the Zona pellucid and the process is called as acrosome reaction which leads to the fusion of the egg membrane with the sperm nucleus. The sperm that fail to reach the egg gets degenerated. Sperm cell survives in the human body up to 2 or 3 days after mating but can be stored for many months or years in frozen state with its viability intact.

Anomalies in Spermatozoa

Certain studies have shown the following abnormalities in the ejaculated spermatozoa (Refer Figure 1.13):

- Spermatozoa with abnormal head, folded tail, connecting piece and sometimes missing tail.
- Immature spermatozoa having two or three tails of equal length, coiled and fused or/ along with round and small head; some time with wide head.
- Immature spermatozoa having two heads along with two fused tails; or one at each tip of the tail.
- Spermatozoa with a triangular head and short, folded tail without an intermediate piece.
- Spermatozoa having no main piece but long intermediate piece along with short tail and rough head.
- Spermatozoa having a small, rounded, aberrant, bacillary and tapering head (Refer Figure 1.13).

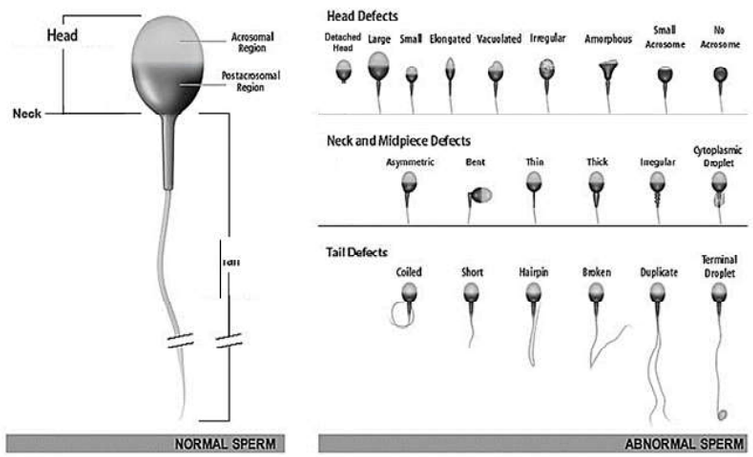

Fig. 1.13 *Diagrammatic Representation of Anomalies of Spermatozoa*

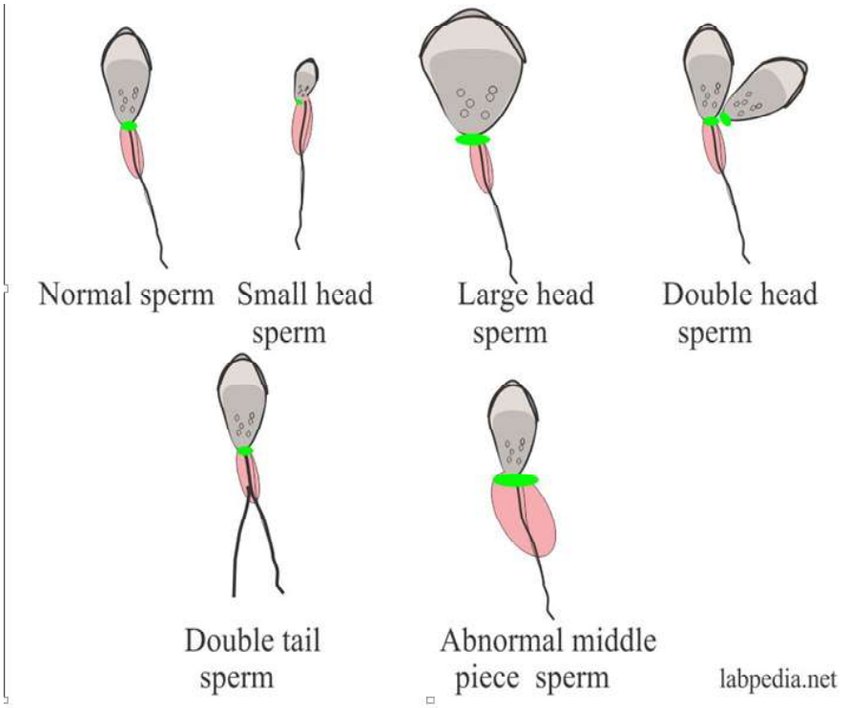

Fig. 1.14 *Sperm Morphology Abnormal Form*

Check Your Progress

6. What is shape of head of a sperm?
7. What is the amount of sperm in an average ejaculation?
8. What is the length of neck of sperm?
9. In how many parts the tail of a sperm divided?

1.5 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Aristotle is the first embryologist known to history.
2. Leeuwenhoek discovered the spermatozoa.
3. Some organisms used extensively in developmental biology are *Danio rerio, Mus musculus, Thales cress,* etc.
4. The factors responsible for regulation of meiotic phase of spermatogenesis are BMPs (Bone Morphogenetic Proteins) and Retinoic Acid.
5. The factor which deficiency leads to abnormal spermatogenesis and altered sperm physiology is FGF-2 (Fibroblast Growth Factor).

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6. The head of the sperm show variation in shape for different animal species. In humans it is flattened, almond-shaped, 4-5µm long and 2-3µm wide.
7. An average ejaculation contains 300,000,000 to 400,000,000 sperm out of which only one enters the egg.
8. The neck is a constriction like structure of about 1µm length and is attached to basal plate, transverse oriented centriole, containing nine segmented column of fibrous materials and continues as outer dense fibers in tail.
9. It has three parts: a middle piece, principal piece, and end piece. A small middle portion of sperm contains mitochondria. The tail of sperm (sometimes called flagellum) is a slender, hair like bundle of filaments which connects with the head and middle portion.

1.6 SUMMARY

- Developmental biology is the field of science that explores the potential of an unorganized fertilized cell to form a definite adult animal. The continuity of life through mysterious embryonic development is an organized process that begins with the formation of microscopic structures called as gametes.
- Gametes are the cells produced by parent generation though the process of gametogenesis (spermatogenesis & oogenesis) and are laden with hereditary information.
- From the day of conception of female till the death of an organism, all the dramatic changes encountered by an organism for its growth and development can be studied only under the field of biology known as Developmental biology.
- The process of progressing from a single cell through the period of establishing organ primordial (the first 8 week of human development) is called the period of embryogenesis or organogenesis.
- The period from this onwards up to the birth is called the fetal period, a time when differentiation continues while the fetus grows and gain weight.
- There are different model organisms in different groups of organisms such as: plants (plants: *Arabidopsis thaliana*; invertebrates: *Drosophila*, *Caenorhabditis elegans*, sea urchins; and vertebrates: ascidians, *Xenopus*, *Danio, Gallus, Mus*).
- Spermatogenesis is the developmental process from germ cell that ends at formation of haploid spermatid, which begins at the puberty and occurs in the seminiferous tubules of the testis.
- The process of spermatogenesis is divided into three major phases: i. Proliferative phase or Multiplicative phase, ii. Meiotic phase, and iii. Post Meiotic phase (Maturation phase).

- Three hormones produced by male gonads directly or indirectly control the process of spermatogenesis. They are testosterone, estradiol and inhibin.
- The deficiency of Fibroblast Growth Factor 2 (FGF-2) leads to abnormal spermatogenesis and altered sperm physiology. Earlier it was revealed that the presence of Fibroblast Growth Factor 2 (FGF-2) and its receptors (FGFRs) in human testis and sperms are involved in spermatogenesis and in motility regulations.
- The different steps of spermiogenesis are distinguished by the morphological appearance of the developing acrosome and the change in the shape of the nucleus.
- The major event occurring during spermiogenesis is the assembly of the sperm flagellum. The central component of the flagellum i.e. microtubule-based axoneme, is assembled soon after the completion of meiosis.
- As spermatids elongate, the accessory structures needed for flagella function (outer dense fibers, fibrous sheath, mitochondrial sheath) are assembled around the central axoneme.
- The final stage of spermiogenesis is known as spermiation and is the process by which the elongated spermatids undergo their final remodeling and get released from the seminiferous epithelium.
- Spermiation is a complex, multi-step process, which is particularly vulnerable to disruption. In case of mice, the last stage of sperm maturation is controlled by *Katnal1* gene.
- The head of the sperm show variation in shape for different animal species. In humans it is flattened, almond-shaped, 4-5µm long and 2-3µm wide.
- Each body cell of a healthy person has 46 chromosomes, which determine the individual's general physical makeup. Head of the sperm is covered by a cap called as acrosome that has enzymes which help sperm to enter an egg.
- An average ejaculation contains 300,000,000 to 400,000,000 sperm out of which only one enters the egg.
- The neck is a constriction like structure of about 1µm length and is attached to basal plate, transverse oriented centriole, containing nine segmented column of fibrous materials and continues as outer dense fibers in tail.
- The middle piece is 5µm long; consist of axonema and dense fibers surrounded by mitochondria.
- Tail have three parts: a middle piece, principal piece, and end piece. A small middle portion of sperm contains mitochondria. The tail of sperm (sometimes called flagellum) is a slender, hair like bundle of filaments which connects with the head and middle portion.

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- The tail is about 50µm long; having thickness of 1µm near the mitochondria which gradually diminishes to less than 0.5µm at the end of tail. The tail helps in sperm cell movement (whips and undulates) so that it can travel to the egg.
- Anandamide is the compound present in the female reproductive track (high concentration near the egg) plays an important role in opening the proton channels.
- The sperm that fail to reach the egg gets degenerated. Sperm cell survives in the human body up to 2 or 3 days after mating but can be stored for many months or years in frozen state with its viability intact.

1.7 KEY WORDS

- **Spermatogenesis:** Spermatogenesis is the developmental process from germ cell that ends at formation of haploid spermatid, which begins at the puberty and occurs in the seminiferous tubules of the testis.
- **Sperm**: Sperm is the male reproductive cell and is derived from the Greek word *sperma* (meaning seed).
- **Spermatozoon**: A uniflagellar sperm cell that is motile is referred to as a spermatozoon.
- **Spermatium**: A non-motile sperm cell is referred to as a spermatium.

1.8 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short Answer Questions

1. Brief a note on proliferative phase.
2. What is post-testicular sperm maturation?
3. Write a short note on hormonal control of spermatogenesis.
4. What are the changes that occur in centrioles during sperm maturation?
5. How does a mitochondria adapt changes while the sperm matures?

Long Answer Questions

1. Discuss in detail about the history, introduction and scope of developmental biology.
2. Explain the process that transforms non-motile spermatids into motile and metabolically active spermatozoa.
3. Describe the hormonal control of the spermatogenesis with illustrations.

4. Diagrammatically explain the process of formation of haploid spermatids with suitable diagrams.
5. Draw the well labeled structure of mammalian spermatozoa and explain its all components?

NOTES

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NOTES

UNIT 2 OOGENESIS, EGG: MEMBRANES AND STRUCTURE

Structure

2.0 INTRODUCTION

In a healthy woman with normal reproductive life, oogenesis is as important as any other life process. The phenomenon of oogenesis is an area that has long been of interests by researchers of various fields such as in medicine, developmental biology, economics, sociology and public policy.

In mammals, small oocytes grow and reach their final size in the ovary where they mature and are prepared to be fertilized. The process of oocyte maturation is a critical event for the developmental potential of an embryo. In domestic animals, such as cattle and pigs, the proportions of oocytes that exhibit the capacity to resume meiosis and support embryonic development increases gradually with increased oocyte diameter. In bovine oocytes, acquisition of meiotic competence does not occur until the antral follicle stage, when the oocyte diameter is greater than 100mm.

The oogenesis is the process of development and maturation of ovum. Mammalian oogenesis differs greatly from the spermatogenesis. Eggs mature through intricate coordination of hormones, paracrine factors and tissue anatomy. The site of oogenesis is female gonads, i.e., ovaries. There are one pair of ovaries (one on each side) are located in lower abdominal cavities of female.

In this unit, you will study about mechanism of oogenesis in female gonads, shape and size of eggs, egg membranes and organization of egg, etc. in detail

2.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand the mechanism of oogenesis in female gonad
- Explain the shape and size of eggs
- Discuss about egg membranes and organization of egg

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2.2 EGG MEMBRANES AND ORGANIZATION OF EGG

In a healthy woman with normal reproductive life, oogenesis is as important as any other life process. The phenomenon of oogenesis is an area that has long been of interests by researchers of various fields such as in medicine, developmental biology, economics, sociology and public policy. Almost four centuries ago, English physician William Harvey said '*ex ovo omnia*', i.e., all that is alive comes from the egg.' During a women's reproductive life span only 300–400 of the nearly 1–2 million oocytes present in her ovaries at birth are ovulated. The process of oogenesis begins with migration of Primordial Germ Cells (PGCs) which results in production of meiotically competent oocytes containing the correct genetic material, proteins, mRNA transcripts and organelles necessary to create a viable embryo. This is a compactly controlled process involving not only ovarian paracrine factors but also signaling from gonadotropins secreted by the pituitary. The gamete production in females is intimately part of the endocrine responsibility of the ovary. If there are no gametes, then hormone production is drastically curtailed. The depletion of oocytes implies depletion of the major hormones of the ovary on contrary in male this is not the case. It means androgen production will proceed normally without a single spermatozoon in the testes.

In mammals, small oocytes grow and reach their final size in the ovary where they mature and are prepared to be fertilized. The process of oocyte maturation is a critical event for the developmental potential of an embryo. In domestic animals, such as cattle and pigs, the proportions of oocytes that exhibit the capacity to resume meiosis and support embryonic development increases gradually with increased oocyte diameter. In bovine oocytes, acquisition of meiotic competence does not occur until the antral follicle stage, when the oocyte diameter is greater than 100mm. The oocytes first acquire the capacity to undergo Germinal Vesicle Breakdown (GVBD). As the follicular diameter increases to approximately 2mm and the oocytes increase in diameter from 110 to 120mm, developmental competency is acquired and the majority of oocytes become capable of supporting fertilization and embryonic development.

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2.2.1 Oogenesis

The oogenesis is the process of development and maturation of ovum. Mammalian oogenesis differs greatly from the spermatogenesis. Eggs mature through intricate coordination of hormones, paracrine factors and tissue anatomy. The site of oogenesis is female gonads i.e. ovaries. There are one pair of ovaries (one on each side) are located in lower abdominal cavities of female. All the events of oogenesis take place inside ovaries. Histological, each ovary is made up of a dense fibrous connective tissue called stroma. It contains blood and lymph vessels, nerves and follicles in various stages of development. The stroma is surrounded by a layer of germinal epithelial cells and internal to it is present a thin layer of connective tissue, called the tunica albuginea. Stroma is further differentiated into outer broad cortex and inner narrow medulla containing only blood and lymph vessels.

The cortex contains ovarian follicles in various stages of development and regressing follicles like corpora lutea and corpora albicans and interstitial cells.

One follicular cell develops to become an ovum and the other cells called discus proligerous around it protect and nourish the ovum. It is attached to one side of the follicle. In a mature Graffian follicle, a fluid-filled cavity appears which separates an outer layer of cells, the membrana granulosa, from the cells of discus proligerous. The follicular cells around the ovum secrete a thick membrane called zona pellucida. It is covered by another membrane of striated columnar cells, called corona radiata. Such a mature follicle is known as a Graffian follicle. The Graffian follicle migrates towards the surface of the ovary and ruptures to release the ovum (ovulation). The release of ovum left a mass of follicular cells surrounding a blood clot. Gradually these follicle cells proliferate rapidly and transform into a yellow colored mass of cells, the corpus luteum, which produces a hormone, progesterone. It is responsible for preparatory changes in the uterus for fertilization and retention of the embryo during gestation (Refer Figure 2.1).

Thus, the corpus luteum remains active throughout the period of embryonic development. If, somehow, no fertilization occurs, it degenerates leaving a scar, called corpus albicans. The process of oogenesis is broadly separated into three phases: Multiplicative phase, Growth phase, and Maturation phase.

Multiplicative Phase or Proliferative Phase

In contrast to spermatogenesis which begins in males at puberty, oogenesis begins in females before they are born. During early fetal development, i.e., 2nd to 7th months of gestation period, Primordial Germ Cell (PGC) migrate from the yolk sac to developing ovaries. These primordial germ cells get differentiated within the ovaries to form Oogonia. The oogonia are diploid (2n) stem cells that divide mitotically to produce roughly 7 million germ cells. Even before birth most of these germ cells degenerate in a phenomenon called Atresia.

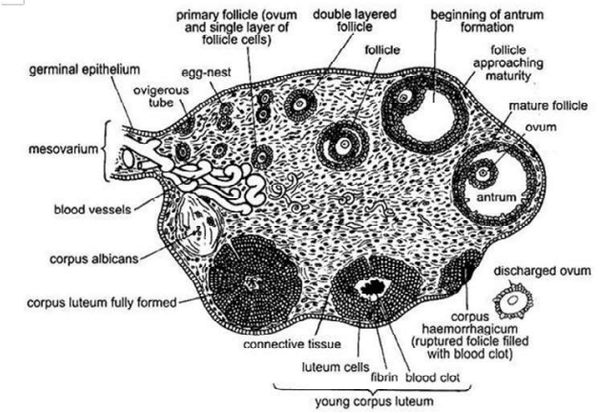

Fig.2.1 *T.S. of Ovary*

Growth Phase

Few of oogonia divide mitotically and develop into larger cells called Primary oocytes. These primary oocytes enter into prophase of meiosis-I during fetal development. After one meiotic division the oocyte arrests itself in the Diplotene stage of first meiotic division. This prolonged arrest in the diplotene stage is also referred as Dictyate resting stage. The oocytes are maintained in the dictyate resting stage by outer layer of ovarian follicular cells. During the arrested stage of development each primary oocyte is surrounded by a single layer of flat follicular cells and entire structure is called as Promordial follicle. Ovarian cortex surrounding primordial follicle consists of collagen fibre and fibroblast like stromal cells. At birth, approximately 200,000 to 20,00000 primary oocytes remain in each ovary. Out of which 40,000 are present at puberty whereas around 400 mature and ovulate during women's reproductive lifetime. Remaining primary oocytes undergo atresia (Refer Figure 2.2).

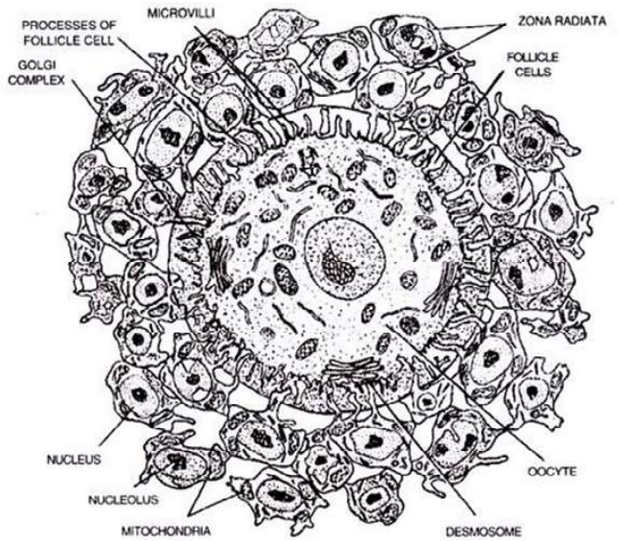

Fig.2.2 *Young Mammalian Oocyte of Surrounded by Follicle Cells*

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Maturation Phase

With onset of puberty, a group of oocytes periodically resume meiosis. At that time Luteinizing Hormone (LH) from the pituitary gland releases the block and permits the oocyte to resume meiotic division. Primary oocyte completes 1st meiotic division producing 2 haploid cells of unequal size each with 23 chromosomes. Smaller cell produced by meiosis are called as first polar body having only nucleus and small amount of cytoplasm. Larger cell are known as secondary oocyte which receive most of cytoplasm. Once a secondary oocyte is formed, it begins meiosis-II which stops at metaphase. Meanwhile, a mature graffian follicles rupture to release secondary oocyte and the process is known as Ovulation. At ovulation, secondary oocyte is expelled into pelvic cavity together with first polar body and corona radiata. Normally, these cells are swept into uterine tube. If sperms are present in the uterine tube, its penetration takes place into the secondary oocyte and meiosis II is resumed. Secondary oocyte splits into two haploid cells of unequal size. Larger cell is ovum or mature egg and second polar body. If there is fertilization then the nuclei of sperm cell and ovum unite forming diploid zygote. If fertilization does not occur, the cells get degenerated. It has been hypothesized that steroids act directly on the oocyte to induce maturation whereas pituitary hormones act through the mediation of follicular tissue (Refer Figure 2.3). Evidences in support of physiological maturation are:

- A positive response to an activation stimulus
- The ability to undergo cleavage and subsequent development

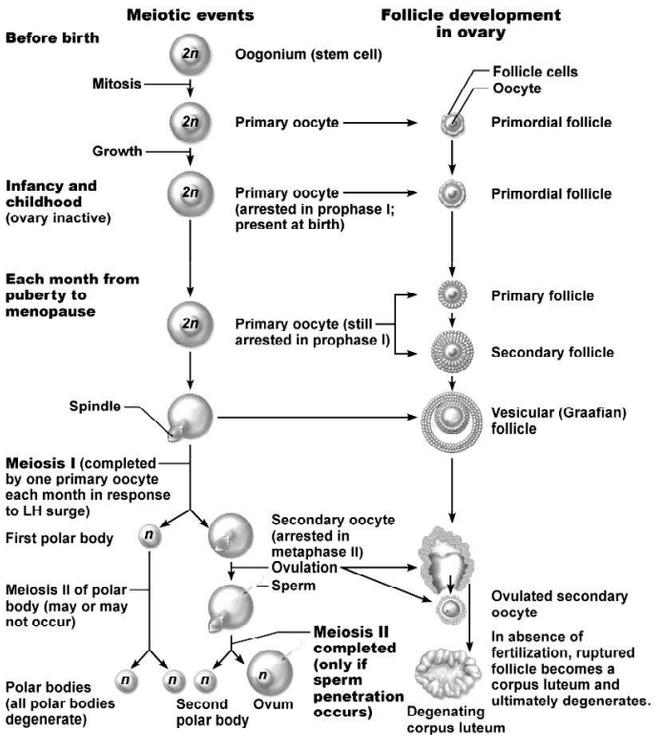

Fig. 2.3 *Schematic Representation of Oogenesis and Follicle Development in Ovary*

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Oogenesis in *Xenopus*

On the basis of anatomy of the developing oocyte, oogenesis in anuran *Xenopus laevis* can be divided into six stages given below:

- The stage-I consists of small colorless oocytes (50 to 100μm) having transparent cytoplasm with clearly visible large nuclei and mitochondrial masses.
- The stage-II oocytes have diameter of 450μm and are white and opaque. The stage I and II are previtellogenic.
- The synthesis of pigment and accumulation of yolk (vitellogenesis) starts at stage-III.
- The vitellogenesis continues through stage-IV (600 to 1000μm), the oocytes grow rapidly and the animal and vegetal hemispheres become differentiated.
- By stage-V (1000 to 1200μm) the oocytes have nearly reached maximum size and yolk accumulation gradually ceases.
- Stage-VI oocytes are characterized by the appearance of an essentially unpigmented equatorial band. They range from 1200 to 1300μm, are post vitellogenic and ready for ovulation.

These stages of oocyte development are correlated with physiological and biochemical changes related to oogenesis in *Xenopus*. Oocytes and their companion somatic cells maintain a very close association throughout oogenesis which is essential for normal oocyte and follicular development. Follicular somatic cells and oocytes are interdependent for development and function. Studies have shown that the regulatory interaction of somatic cells and oocytes; however, is bidirectional. The oocyte participates in the proliferation, development and functions of follicular somatic cells and also secretes factors that facilitate the cumulus cells to synthesize hyaluronic acid and undergo cumulus expansion against hormonal stimulation (Refer Figure 2.4).

Oogenesis in Mouse

Histological and cytological study by the squash technique in albino mice of the street strain ovaries from the 10th day of fetal life until the 5th day after birth shows the following pattern of oogenesis:

- **10th–13th Day**: The gonads get differentiated into male and female gonads.
- **14th Day**: Germ cell stops oogonial division and enters meiosis.
- **14th–15th Day**: Leptotene and zygotene stages are visible together.
- **16th Day**: Pachytene nuclei appear in large number.
- **17th-18th Day**: All oocytes enter in pachytene stage which either in diplotene or gets degenerated.
- **18th–19th Day:** Late diplotene stage till 4th or 5th day of life.

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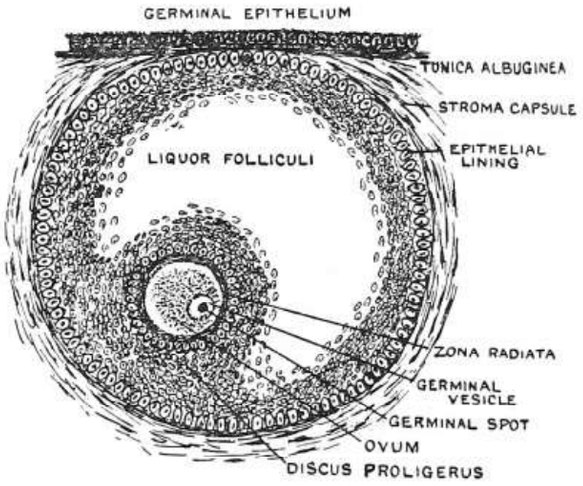

Fig. 2.4 *Mature Mammalian Ova*

Hormonal Control of Oogenesis

The process of oogenesis is controlled by the interaction of hormones released from hypothalamus and adenophysis (anterior pituitary) with the hormones secreted from reproductive tissue ad organs (Refer Figure 2.5).

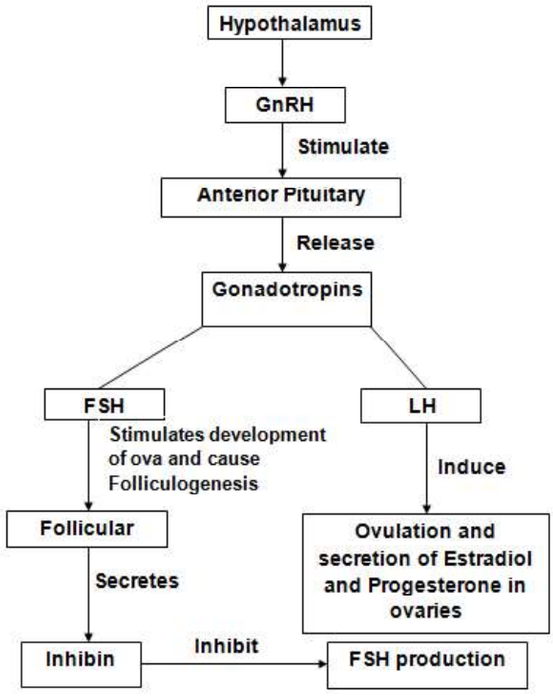

Fig.2.5 *Hormonal Control of Oogenesis*

2.2.2 Organization of Egg

The organization of egg can be divided into egg cortex, egg yolk, egg membranes, egg shell and egg pigments (Refer Figure 2.6).

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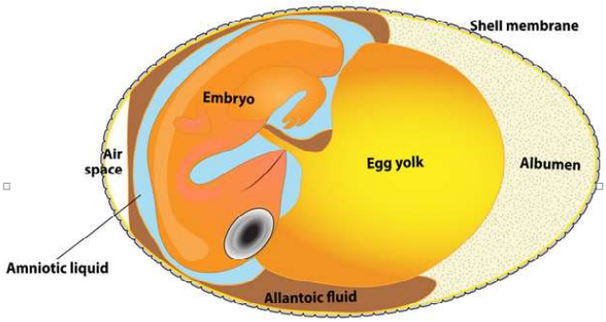

Fig. 2.6 *Egg and its Parts*

Egg Cortex

The egg cortex is an assemblage of plasma membrane, extracellular coat and surface skeleton to which a characteristic set of cytoskeletal elements, organelles and macromolecules adheres. It acts as functional unit during the reorganization that follows fertilization. Different physical properties of the egg cortex such as morphology, stiffness, thickness, gets changed during the maturation of egg and its fusion with sperm i.e. fertilization. In most of the eggs beneath the cell membrane there is a thin layer made up of gel-like cytoplasm called as cortex. The cortex is a semi-rigid gel having higher viscosity and the cytoplasm present in this region is comparatively thicker as compared to the internal cytoplasm, containing high concentration of globular actin molecules.

During the process of fertilization these globular actin molecules polymerize to form long cables called as microfilaments which plays a crucial role during cell division as it helps in the formation of the mitotic apparatus. The cortical granules present within egg cortex are membrane-bound structures formed from Golgi bodies and contains proteolytic enzymes. Due to presence of these proteolytic enzymes cortical granules are regarded as the homologues of the acrosome vesicles present in the sperm head. Along with the digestive enzymes cortical granules also contains the mucopolysaccharides, adhesive glycoproteins and hyalin proteins which helps in the process of fertilization. Egg cortex also contains pigment granules in case of amphibians which contains dark brown or black pigment that imparts black or brown color to the egg. Human ovum lacks cortical granules.

Functions of Egg Cortex

- Mucopolysaccharides play an important role in preventing the polyspermy during fertilization, i.e., entry of more than one sperm.
- Hyalin and Glycoproteins surrounds early embryo and provide support during the process of cleavage.
- Small egg projections (microvilli) help in the entry of sperm into the ovum.

Egg Yolk

Inside the eggs of various animals yellow colored substance is present which is called as egg yolk (also known as vitellus). However yolk word is not a definite chemical substance rather than it is a morphological term used to signify the reserve food material present inside the egg. Chemically, yolk is a lipoprotein that consists of proteins, neutral fats, phospholipids, carbohydrates along with very small amount of glycogen.

Composition of Egg Yolk

Yolk is a heterogeneous substance, i.e., mixture of various components and its composition varies from animal to animal. When the yolk contains more protein content as compared to other components, it is called as Protein yolk; whereas when yolk contains proportionality more phospholipids and fats, it is called as Fatty yolk. In case of oviparous and ovoviviparous animals both types of yolks are present. Protein yolk is the main food reserve material in the eggs of many invertebrates and in lower chordates such as *Amphioxus*. Fatty yolk is abundantly formed in eggs of bony fishes, reptiles, birds, etc.

Proteins: 17%

Lipid and neutral lipids: 65%

PC: 8.3%

PE: 1.4%

Sphingomyelin: 2.5%

Phosphatidylinositol: 0.5%

Cholesterol: 5%

Carotenoids (carotenes), Xantophylls (lutein, zeaxanthin): 0.3%

Proteins (17%)

Lipovitellins (a- and b-): 69%

- Lipovitellins-a: 58%
- Lipovitelins-b: 11%

Livetins: 12%

- Livetin-a (serum albumin): 4%
- Livetin-b (glycoprotein): 5%
- Livetin-g (g-globulin): 2%
- Phosvitin: 1%

Apo/ low-density lipoproteins: 2%

Forms of Yolk

The yolk occurs in two principal forms in eggs, i.e., Granular yolk, and Platelet yolk.

- **Granular Yolk:** Their yolk is present in the form of fine granules which are uniformly distributed throughout the egg. Examples are eggs of invertebrates and lower chordates such as *Amphioxus*.
- **Yolk Platelets:** In case of vertebrate eggs yolk is present in form of large, flattened and ovoid membrane organelles called as yolk platelets. These yolk platelets consist of Phosvitin and Lipovitellin. Lipovitellin is also a protein and contains 17% of the lipid content. In case of Yolk Platelet, yolk structures are arranged in crystalline lattice with hexagonal packing.

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Synthesis of Yolk

Yolk synthesis is of two types:

- **Autosynthesis:** In this type of synthesis the components essential for the synthesis of yolk are present inside the oocyte itself. Egg is not dependent upon other factors for yolk to get synthesized.
- **Heterosynthesis:** In this type of synthesis the components needed for the synthesis of the yolk are present inside the egg rather gets transported from the other organs of the female body. Mainly yolk is synthesized in the liver of the female and present in the insoluble form and gets transported through circulation to the developing follicular cells that surrounds the ovum to form yolk platelet and the granules gets deposited in the ooplasm. In vertebrates the yolk synthesis is hetero-synthetic in nature. Major precursor of yolk in this kind of synthesis is Vitellogenin protein secreted by the Oestrogens and activity is regulated by the Vit gene.

Functions of Yolk

- Yolk acts as a source of energy to developing embryo.
- Yolk provides raw material needed for the synthesis of molecules required for the elongation of embryonic body.
- The amount of yolk greatly determines the pattern of cleavage.
- It determines the development of organism (direct/ indirect).
- Yolk greatly influence the size of egg and differentiation of ooplasm.
- Yolk controls the morphogenetic movements of blastomeres during gastrulatio.

2.2.3 Egg Membranes

In most of the eggs layered structures or the protective formations are present covering the egg. Structure of these protective coverings varies from species to species and such protective coverings are known as egg membranes. These are fluid protein membranes present around the egg and are produced either by the egg itself or by the follicular cells of the ovary or by the genital duct (oviduct) of the female. These membranes protect egg from physical injury, desiccation, microorganisms or parasites and are also used by developing embryos as nutrient matter. The separation of the membrane from the surface of the egg during fertilization plays a crucial protective role against penetration by too many spermatozoa (Cortical reaction). These membranes are absent in eggs of some animals which show amoeboid movement, for example, sponges and certain coelenterates.

Types of Egg Membranes or Egg Coats

There are three main types of egg membranes:

- Primary Egg Membrane
- Secondary Egg Membrane

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- Tertiary Egg Membrane

Primary Egg Membrane

It is secreted by the egg itself during the process of oogenesis. It is formed outside the plasma membrane in the space occupied by interdigitating microvilli between oocyte and follicle cells.

They are closely attached to the surface of the egg. The primary membranes are named differently in the different animals. Different kinds of egg membranes are as follow:

- **Plasma Membrane:** It is the membrane covering the egg immediately over it and found in all the eggs. Structurally, it resembles to the plasma membrane of a cell and closely attached to the surface of the egg. The primary membranes are named differently in different animals.
- **Vitelline Membrane:** It is very thin and transparent and is closely attached to the plasma membrane of the egg. It is commonly found in egg of amphioxus and mollusks, echinoderms, amphibians, birds, etc. It is formed of mucopolysacharide and fibrous protein. The space formed between it and the plasma membrane is called perivitelline space, filled with a fluid known as perivitelline fluid
- **Corona Radiata:** The egg of the shark *Scyllium canicula*, has two primary membranes produced by the surface ooplasm. The outer membrane is the vitetline membrane and the inner membrane is zona radiata having a radiating appearance. The eggs of teleost fishes are also covered by zona radiata.
- **Zona Pellucid:** All mammalian eggs are surrounded by a membrane called zona pellucida and crona radiata. It is so named because it gives a striated appearance under the microscope. The striations are due to the presence of microvilli and macrovilli (desmosomes) in this zone. The microvilli are present on the surface of the egg and microvilli are produced by follicular cells which protrude into the zona pellucida.
- **Jelly Envelope:** This kind of primary egg membrane is found in the eggs of echinoderms and many marine invertebrates. In this case, the egg membrane is thicker as compared to others and is called as Jelly coat.

Secondary Membranes

The secondary membranes are produced by the follicle cells (cells found around the developing oocytes) of the ovary and are usually tough and impermeable; preset outside the primary egg membrane. The secondary membranes are as follows:

- **Chorion:** This is a common outer covering in the eggs of insects, ascidians and cyclostomes (*Myxine*). It is found outside the vitelline membrane. As the chorion is tough and impermeable, it is provided with one or more openings called micropyles through which the sperms enters the egg.

- **Corona Radiata**: This membrane is formed of a layer of follicle cells where the cells are radially arranged around the zona pellucida. It is found in mammalian eggs.

Tertiary Membranes

The tertiary membranes are produced either by oviduct or some maternal tissues other than ovarian tissues.

- **White Albumen:** It is found outside the vitelline membrane in the egg of hen, oviparous elasmobranches, and horny egg capsule (secreted by egg shell gland of oviduct) in the eggs of shark. It is formed of three layers: an inner less dense albumen, a middle dense albumen and an outer less dense albumen. The albumen is formed of water and proteins.
- **Shell Membrane:** The shell membrane is formed around the albumen in the egg of hen. It is a double membrane in which the two membranes adhere closely and are separated by an air space at the blunt end of the egg. This membrane is formed of keratin.
- **Shell:** The shell is the outer covering of the eggs of land animals and is formed of calcium carbonate. It is white or brown in colour and contains as many as 7000 minute pores. These pores are 0.04 to 0.05 mm in diameter and are filled with a proteinous substance called as collagen.
- **Jelly Coat:** The amphibian eggs are surrounded by a gelatinous covering called as jelly coat.
- **Mermaid's Purse**: It is the egg in case of some cartilaginous fishes. It is a protective hard shell secreted by the shell glands present in the oviduct. The shape of the purse varies from group to group.

Generally, it is rectangular in shape and the corners of the shell are drawn out into four long twisted elastic filaments which serve to attach the eggs to sea weeds. In dog-fish *Chiloscyllium*, development is completed within this purse.

Functions of Egg Membrane

- The primary function of egg membrane is to provide protection to the developing embryo from physical injury and chemical gradations, temperature variation, radiations, pH etc.
- In hemichordates, it prevents self-fertilization and prevents polyspermy.
- In amphibians egg membranes provide buoyancy to the egg.
- In reptiles' and birds, egg white acts as an additional source of nourishment to the developing embryo.
- In mammalian egg zona pellucida plays a significant role I mitotic divisions of the zygote post fertilization.
- It prevents unwanted fusion of two eggs rarely produced at the same time, and helps in cortical reaction.

NOTES

Egg Shell

The egg shell, which is a primary packaging container and also a microbial barrier, is of great importance to poultry producers. The eggshell layers are deposited in a precise order as the egg descends through the highly differentiated parts of the oviduct. The hen egg shell is composed of three main layers; each having its own complex morphology, i.e., the cysteine-abundant proteinaceous shell membrane, the mineralized hard complex layer and the outer non-mineralized cuticle. The shell membrane is synthesized over a period of 1.0 to 2.0h, when the immature egg travels through the proximal isthmus. The mineralized multilayered complex layer is formed in the distal isthmus and shell gland over an approximately 19 to 20h time period. The cuticle is deposited onto the egg shell in the uterus 1.5 to 2.0h before oviposition. The egg shell is composed mainly of calcite but a thin layer of hydroxyapatite is also present in the inner cuticle.

Egg Shell Pigments

The study of egg shell pigments has a long history and the major pigment extracted from brown egg shells was initially named oorhodein. Later, it was confirmed that the brown pigment is protoporphyrin IX. Protoporphyrin IX belongs to a group of families of biologically active tetrapyrrole compounds. Structurally, protoporphyrin IX is a tetrapyrrole ring containing a highly conjugated planner and a rigid macrocycle consisting of four pyrrole rings connected by methane groups. The nomenclature of this tetrapyrrole compound often includes a numerical suffix such as IX, which refers to the position of the side chain. In addition to protoporphyrin IX, biliverdin, coproporphyrin and uroporphryin were also identified from domestic hen eggshell and shell gland. However, the dominant egg shell pigment in brown-egg laying hens is protoporphyrin IX, with traces of other porphyrins. The complex nature of egg shell color in laying hens is still under investigation and the fact that certain hens lay brown eggs, others lay white and some lay even blue-shelled eggs has long indicated a genetic basis for shell color, possibly involving sex linked genes. More recent studies have confirmed the high heritability of shell color.

Brown eggshell color has been positively correlated with some shell characteristics such as shell strength and hatchability. Apart from quality functions, in the presence of light, brown pigment has shown bactericidal activity against certain gram positive bacteria. Major pigment in egg shells of brown egg laying hens is protoporphyrin IX, but traces of biliverdin and its zinc chelates are also present. The pigment appears to be synthesized in the shell gland. The protoporphyrin IX synthetic pathway is well defined but precisely where and how it is synthesized in the shell gland of the brown-egg laying hen is still ambiguous. The pigment is deposited onto all shell layers including the shell membranes, but most of it is concentrated in the outermost layer of the calcareous shell and in the cuticle.

Recently, the genes that are involved in pigment synthesis have been identified. Genetic control of synthesis and deposition of brown pigment in the commercial laying hen is not fully understood. The brown coloration of the shell is an important shell quality parameter and has a positive influence on consumer preference. The extent of pigment deposition is influenced by the housing system, hen age, hen strain, diet, stressors and certain diseases such as infectious bronchitis. The color of the eggshell is assumed to be controlled by several genes that encode proteins and enzymes, thereby regulating the production and deposition of pigment into the shell.

Check Your Progress

1. Name the phenomenon which encompasses the degeneration of germ cells before the birth of female baby?
2. At which stage oocyte arrest itself in the Diplotene stage after the completion of first meiotic division?
3. Write an example of an organism whose eggs are having the covering of egg albumen.
4. Write the composition of yolk platelet.
5. How many ova synthesize during one complete cycle of oogenesis?

2.3 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Atresia is the phenomenon which encompasses the degeneration of germ cells before the birth of female baby.
2. Dictyate Resting Stageis the stage at which oocyte arrest itself in the Diplotene stage after the completion of first meiotic division.
3. Oviparous Elasmobranches, Sharks is an example of an organism whose eggs are having the covering of egg albumen.
4. Phosvitin and Lipovitellin includes the composition of yolk platelet.
5. One ova synthesize during one complete cycle of oogenesis.

2.4 SUMMARY

- English physician William Harvey said '*ex ovo omnia*' i.e. 'all that is alive comes from the egg.'
- The oogenesis is the process of development and maturation of ovum. The site of oogenesis is female gonads, i.e., ovaries.

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- The process of oogenesis is broadly separated into three phases: Multiplicative phase, Growth phase, and Maturation phase.
- At birth, approximately 200,000 to 20,00000 primary oocytes remain in each ovary. Out of which 40,000 are present at puberty whereas around 400 mature and ovulate during woman's reproductive lifetime. Remaining primary oocytes undergo atresia.
- The process of oogenesis is controlled by the interaction of hormones released from hypothalamus and adenophysis (anterior pituitary) with the hormones secreted from reproductive tissue ad organs.
- The organization of egg can be divided into egg cortex, egg yolk, egg membranes, egg shell and egg pigments.
- Inside the eggs of various animals yellow colored substance is present which is called as egg yolk (also known as vitellus).
- Plasma membrane is the membrane covering the egg immediately over it and found in all the eggs. Structurally, it resembles to the plasma membrane of a cell and closely attached to the surface of the egg. The primary membranes are named differently in different animals.
- Corona radiata is the egg of the shark *Scyllium canicula*, has two primary membranes produced by the surface ooplasm. The outer membrane is the vitetline membrane and the inner membrane is zona radiata having a radiating appearance. The eggs of teleost fishes are also covered by zona radiata.
- All mammalian eggs are surrounded by a membrane called zona pellucida and crona radiata. It is so named because it gives a striated appearance under the microscope.
- Jelly envelope is a kind of primary egg membrane is found in the eggs of echinoderms and many marine invertebrates. In this case, the egg membrane is thicker as compared to others and is called as Jelly coat.
- Chorion is a common outer covering in the eggs of insects, ascidians and cyclostomes (*Myxine*). It is found outside the vitelline membrane. As the chorion is tough and impermeable, it is provided with one or more openings called micropyles through which the sperms enters the egg.
- Corona Radiata is the membrane is formed of a layer of follicle cells where the cells are radially arranged around the zona pellucida. It is found in mammalian eggs.
- The shell membrane is formed around the albumen in the egg of hen. It is a double membrane in which the two membranes adhere closely and are separated by an air space at the blunt end of the egg.
- The shell is the outer covering of the eggs of land animals and is formed of calcium carbonate. It is white or brown in colour and contains as many as 7000 minute pores.

- In hemichordates, egg membrane prevents self-fertilization and prevents polyspermy.
- In amphibians egg membranes provide buoyancy to the egg.
- In reptiles' and birds, egg white acts as an additional source of nourishment to the developing embryo.
- In mammalian egg zona pellucida plays a significant role I mitotic divisions of the zygote post fertilization.
- The egg shell, which is a primary packaging container and also a microbial barrier, is of great importance to poultry producers.
- The eggshell layers are deposited in a precise order as the egg descends through the highly differentiated parts of the oviduct.

2.5 KEY WORDS

- **Oogenesis:** The oogenesis is the process of development and maturation of ovum.
- **Primary oocytes:** Few of oogonia divide mitotically and develop into larger cells called Primary oocytes.
- **Chorion:** It is a common outer covering in the eggs of insects, ascidians and cyclostomes (*Myxine*).
- **Corona radiata**: This membrane is formed of a layer of follicle cells where the cells are radially arranged around the zona pellucida.
- **White albumen:** It is found outside the vitelline membrane in the egg of hen, oviparous elasmobranches, and horny egg capsule (secreted by egg shell gland of oviduct) in the eggs of shark.
- **Shell membrane:** The shell membrane is formed around the albumen in the egg of hen.
- **Shell:** The shell is the outer covering of the eggs of land animals and is formed of calcium carbonate.
- **Jelly coat:** The amphibian eggs are surrounded by a gelatinous covering called as jelly coat.
- **Mermaid's purse**: It is the egg in case of some cartilaginous fishes. It is a protective hard shell secreted by the shell glands present in the oviduct.

2.6 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short Answer Questions

1. What is oogenesis?
2. Brief a note on multiplicative phase or proliferative phase.

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3. Give short note on oogenesis in *Xenopus*.
4. Brief a note on egg cortex.
5. Write a short note on composition of egg yolk.
6. How does synthesis of yolk occur?

Long Answer Questions

1. Describe in detail the mechanism of oogenesis in different animals with suitable illustrations.
2. Enlist some of the major changes that take place after the growth phase of oogenesis with brief description.
3. Explain the composition, forms and functions of yolk to the developing embryo.
4. Write short note on the following:
   - Egg cortex
   - Egg shell pigments
   - Egg membranes
5. Explain the hormonal control of oogenesis with necessary diagram.

2.7 FURTHER READINGS

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UNIT 3 EGGS: MATURATION AND CLASSIFICATION

NOTES

Structure

3.0 INTRODUCTION

The maturation is the process that takes the oocyte from the Germinal Vesicle stage to a stage of meiosis at which the egg can be fertilized or in other words it's a process of making the oocyte ready for the fertilization. After the complete development of oocyte, it enters into second phase where resumption of meiosis takes place for the maturation of egg. Varying with different species, the growth of oocyte arrests at different stages of cell cycle. In ascidians and some Molluscs the growth arrest at Metaphase 1 and in case of Amphibians and Mammals the growth arrests at Metaphase 2 stage of cell cycle. In case of reptiles and birds during the vitellogenic phase of oogenesis, the oocyte attains its full growth and oocyte become ready for the next phase of oogenesis, i.e., resumption of meiotic division which is essential for the final maturation of the oocyte.

The process in which prophase I arrested oocyte resume their meiosis is called as maturation. The term maturation is generally used to describe the completion of meiosis as two successive meiotic divisions occur in the animal oocyte, involving the nuclear and cytoplasmic changes. Ovum maturation or meiotic maturation and the ovulation forms the terminal stages of oogenesis, i.e., formation of female germ cells. Both nuclear and cytoplasmic changes occur during oocyte's final maturation, which have received relatively much more attention in other animal species than in reptiles and birds.

In this unit, you will study about how maturation of egg occur, mechanism of maturation, classification of eggs and egg polarity and symmetry in detail.

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3.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand how maturation of egg occur
- Explain the mechanism of maturation
- Define the classification of eggs
- Describe the egg polarity and symmetry

3.2 MATURATION: GENERAL INTRODUCTION

The maturation is the process that takes the oocyte from the Germinal Vesicle stage to a stage of meiosis at which the egg can be fertilized or in other words it's a process of making the oocyte ready for the fertilization. After the complete development of oocyte, it enters into second phase where resumption of meiosis takes place for the maturation of egg. Varying with different species, the growth of oocyte arrests at different stages of cell cycle. In ascidians and some Molluscs the growth arrest at Metaphase 1 and in case of Amphibians and Mammals the growth arrests at Metaphase 2 stage of cell cycle. In case of reptiles and birds during the vitellogenic phase of oogenesis, the oocyte attains its full growth and oocyte become ready for the next phase of oogenesis, i.e., resumption of meiotic division which is essential for the final maturation of the oocyte. For the successful fertilization, the oocyte maturation is pre-requisite event that comprises of the breakdown of germinal vesicle, chromosomal condensation and expulsion of first polar body in case of vertebrates. The breakdown of germinal vesicle leads to the intermixing of nuclear components with the surrounding cytoplasm. However, in case of animals which do not have haploid generation, the completion of meiosis is immediately followed by the fusion of gametic nuclei, i.e., fertilization. In case of reptiles and birds the first meiosis gets arrested at the diplotene stage of Prophase I of Meiosis I, where the metabolic activities of oocyte is very high and also there is enormous increase in the oocyte. In all animals (amphibians, fishes, reptiles, birds & mammals) the growth of oocyte gets arrested at prophase I and resumes the meiosis at the end of their growth period.

3.3 MATURATION OF EGG

The process in which Prophase I arrested oocyte resume their meiosis is called as maturation. The term maturation is generally used to describe the completion of meiosis as two successive meiotic divisions occur in the animal oocyte, involving the nuclear and cytoplasmic changes. Ovum maturation or meiotic maturation and the ovulation forms the terminal stages of oogenesis (Refer Figure 3.1), i.e., formation of female germ cells. Both nuclear and cytoplasmic changes occur during

oocyte's final maturation, which have received relatively much more attention in other animal species than in reptiles and birds. Ovulation and maturation in vertebrates, including reptiles and birds are closely linked by common systemic factors which appear to correlate with the physiology of the ovary and the oocytes or shows that ovum maturation and ovulation in vertebrates are casually linked in some manner. It is now well established that administration of gonadotrophins to female fish, amphibians and mammals having fully grown oocytes in their ovaries, causes oocyte maturation with concomitant ovulation.

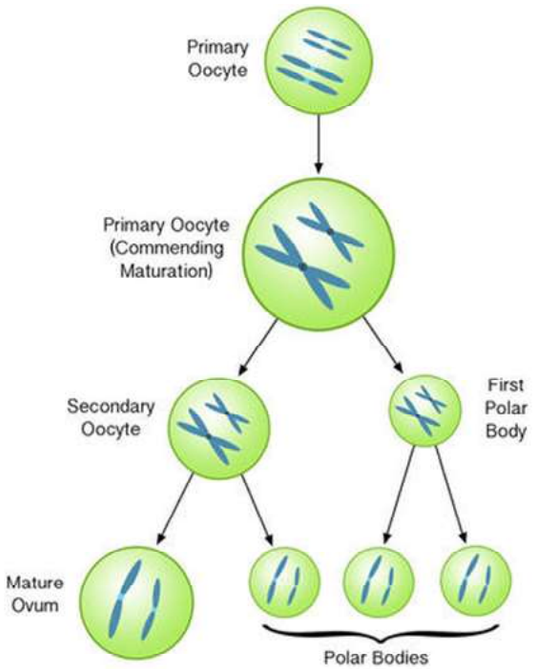

Fig. 3.1 *Oogensis*

In vertebrates, gonadotrophins are secreted by the pituitary gland or hypophysis. Two different gonadotrophins, such as LH and FSH have been distinguished in mammals, birds and reptiles except squamates but only one Gonadotrophic Hormone (GTH) is believed to occur in fish. During recent years, the effects of gonadtrophic hormones, steroid hormones and various other chemical substances on oocyte maturation both in-vivo and in-vitro conditions have been extensively studied in fish, amphibians and mammals but very little or no work on these lines has been carried out on reptiles and birds. The results of various in vivo and in vitro studies on oocyte maturation in fish, amphibians and mammals have suggested that gonadotrophins act primarily on the follicle granulosa cells to induce maturation of oocytes in their ovarian follicles (Refer Figure 3.2).

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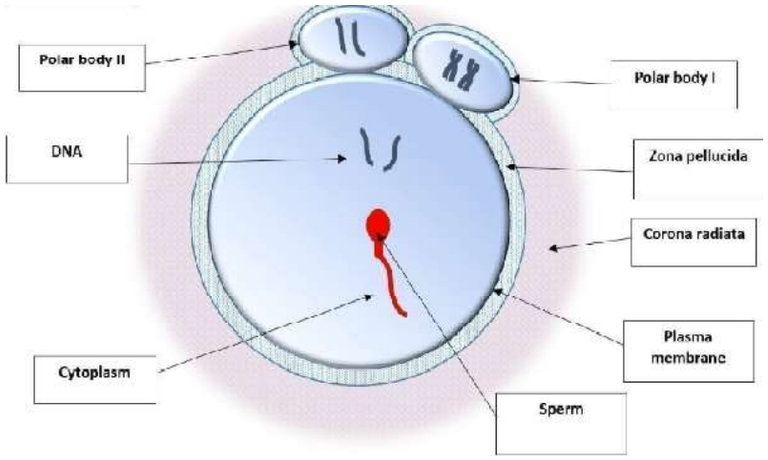

Fig. 3.2 *Fertilized Oocyte*

The cell-to-cell interactions within the vertebrate ovary are of great significance to harmonize the morphological and functional differentiation of the various cell types (theca cells, follicle cells and oocyte) in order to ensure the proper development, differentiation and ovulation of mature eggs which are able to fertilize and develop normally. Besides producing steroid and non-storoid hormones, nutrients and possibly the informational molecules for the oocyte, the follicle cells are also believed to be the source of oocyte maturation-inhibiting factor(s); as suggested for fish and mammals. Actually, a series of complex interactions between pituitary gonadotrophin(s) and various steroid hormones secreted by the follicle wall regulate several aspects of follicular growth and ovum maturation in vertebrates. As a result of the action of gonadotrophins on follicle cells, the steroid hormone, possibly progesterone, is believed to be synthesized and released. This steroid hormone (progesterone) is known to act directly on the oocyte. In response to progesterone, oocytes undergo a series of morphological and biochemical changes, resulting in the formation of mature eggs capable of being fertilized.

3.3.1 Maturation in Different Vertebrates

Following are the maturations for different vertebartes:

In Frog

In the frog *Rana pipiens*, duration of oogenesis is three years. The first two years of the oogenesis involves the gradual increase in the size of oocyte and in the last year of the oogenesis there is rapid accumulation of the yolk inside the egg which causes the swelling of the egg and help in attaining the big characteristic size. In case of frog, the eggs mature in yearly batches in which the first cohort of the eggs just matures immediately after the metamorphosis and remaining eggs matures yearly. The accumulation of the yolk takes place when the oocyte reaches the diplotene stage of the meiotic prophase.

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In Rabbit

Ovulation occurs only after mating and is stimulated by the physical stirring of the cervix (mating). This physical stimulation of cervix trigger the Hypothalamus to release the Gonadotrophin Releasing Hormone (GnRH) which further stimulate anterior pituitary to release the gonadotrophin (FSH & LH). Further these gonadotrophin signals the ovary to reinitiate the process of meiosis followed by the process of ovulation, i.e., release of eggs. This mechanism of maturation of oocyte ensures the fertilization as process of ovulation is stimulated only by the mating.

In Dog and Other Mammals

There is periodic ovulation pattern that is ovulation occurs only at specific period in a year. This kind of ovulation is called as Estrus /heat period/oestrus. During this, they are in heat period which means they are ready to accept or mate with a male. However, female dog is diestrous by nature which means it goes into heat twice in one year. For the initiation of the ovulation, environmental factors play crucial role such as amount and type of light perceived by the organism. Any environmental factor that triggers the hypothalamus to release the Gonadotrophin Releasing Hormone (GnRH) further stimulate anterior pituitary to release the gonadotrophin (FSH & LH). This gonadotrophin stimulates the ovarian follicle cells and after the stimulation of ovarian follicular cells the process of meiosis gets resumed along with the estrogen which determines the pattern of mating behavior also.

3.3.2 Mechanism of Maturation

Below are the steps that occur in the mechanism of maturation:

Resumption / Reinitiation of Meiosis-I

In amphibian oocyte, the resumption of the meiosis I requires the progesterone. Hormone progesterone is secreted by the Follicular cells in response to the gonadotropic hormone secreted by the anterior lobe of pituitary. Within 6 hours of progesterone stimulation, the nuclear membrane dissolves and this is known as Germinal Vesicle Breakdown (GVBD) as the nucleus in prophase is called as Germinal Vesicle. Along with this, other events that take place are the retraction of the microvilli, disintegeration of the nucleoli, contraction of chromosome and the chromosomal migration towards the animal pole. Promptly first meiotic division and the ovulation (i.e., release of ovum) take place from the ovaries, when the egg is released from the ovaries and is in the second meiotic metaphase stage.

Germinal Vesicle Breakdown (GVBD) by Progesterone

M phase of the cell cycle in both (Meiosis and Mitosis) is regulated by Mitosis Promoting Factor or Maturation Promoting Factor (MPF). MPF has two units P^{34} and Cyclin B. P^{34} protein is a cyclin dependent Kinase and its activity is dependent on Cyclin. Since all the components of MPF are present in the amphibian

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oocyte, it is generally thought that Progesterone converts inactive MPF into active MPF. Mediator of the progesterone signal is the C-moss protein. Progesterone reinitiates the meiosis by causing polyadenylation of maternal C-moss mRNA that has been stored in the cytoplasm, which is then translated into a 39-KDa phosphoprotein (C-moss protein). C-moss protein which is only detectable during oocyte maturation is destroyed quickly upon fertilization. This C-moss protein phosphorylates the P^{34} subunit of MPF and also activates the phosphorylation cascade. Now the activated MPF allows the germinal vesicle breakdown and chromosomes to divide. If the translation of C-moss mRNA is inhibited by injecting C-moss antisense mRNA into ooocyte then there will not be any germinal vesicle breakdown and hence resumption of meiosis I will not take place.

Resumption of Meiosis-II

After the first meiotic division the oocytes again arrest its division at metaphase of second meiotic division, this block or arrest is caused by the CSF (Cytostatic Factor). CSF is a complex of proteins that includes C-moss, Cyclin Dependent Kinase 2(Cdk2) and MAP Kinase, Erp1 (an active protein which is synthesized immediately after first meiotic division). Phosphorylation of Erp1 block the degradation of cyclin by Anaphase Promoting Complex and the arrest at the metaphase stage is broken by fertilization. If fertilization occurs, calcium ion flux activates Calcium Binding Protein – Calmodulin which activates two enzymes and these two enzymes further inactivates CSF, Calmodulin dependent Protein Kinase 2 (Cam PK2) and Calpain2 (calcium dependent Protease) inactivates C-moss (Refer Figure 3.3).

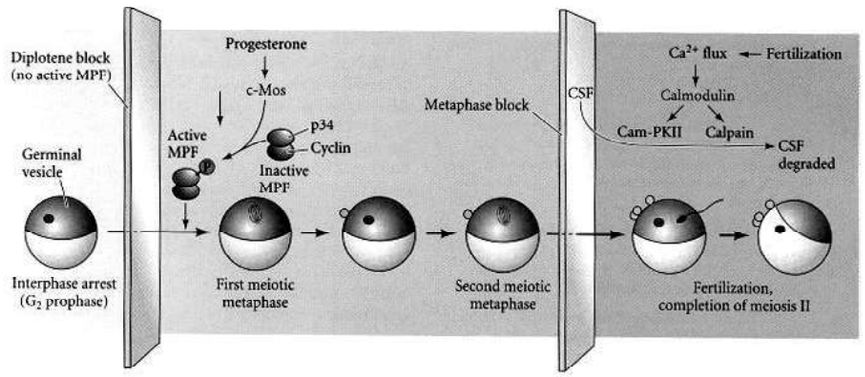

Fig. 3.3 *Oocyte Maturation in* Xenopus.

3.3.3 Classification of Eggs

The eggs are classified in different categories on the basis of amount of yolk, distribution of yolk, shell, etc. (Refer Figure 3.4 and 3.5).

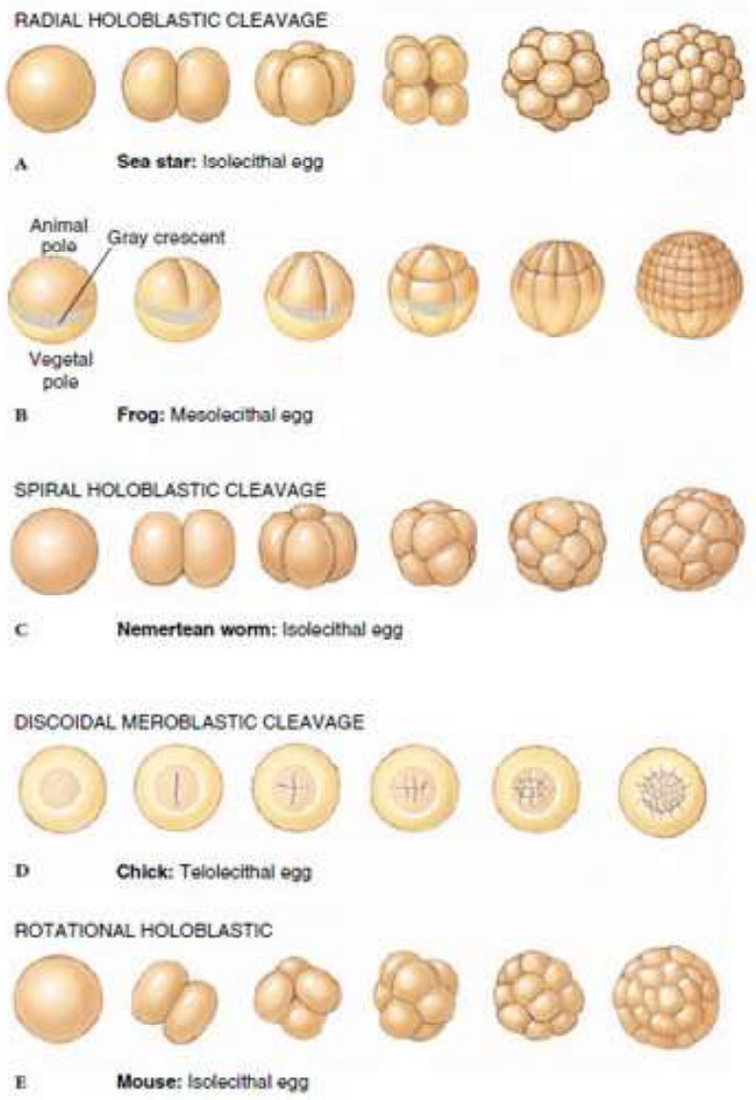

Fig. 3.4 *Classification of Eggs*

On the Basis of the Amount of Yolk

- **Alecithal Egg**: When the egg contains no yolk, it is called alecithal egg, for example, eggs of eutherian mammals.
- **Microlecithal or Meiolecithal Egg**: When the egg contain very small or negligible amount of yolk then the egg is known as Microlecithal egg. Romer and Balinsky named these eggs as oligolecithal eggs, for example, in Amphioxus, Tunicates, etc.
- **Mesolecithal Egg**: In amphibian, Dipnoi and Petromyzon the amount of yolk present is moderate and is not high. Hence these eggs are also named as mesolecithal eggs.

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- **Macrolecithal or Megalecithal or Polylecithal Egg**: Egg that contains large amount of yolk is known as macrolecithal or megalecithal or polylecithal egg, for example reptiles, birds, Prototheria (Monotremata); Egg laying mammals.

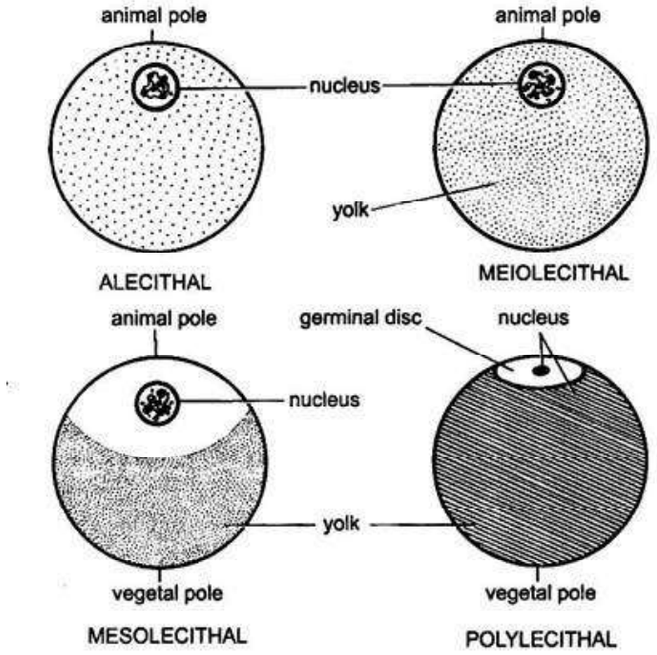

Fig. 3.5 *Types of Ova in Chordates based on Amount of Yolk*

On the Basis of the Distribution of Yolk

Following are the classification of eggs on the basis of yolk: (Refer Figure 3.6)

- **Isolecithal or Homolecithal Egg**: In isolecithal eggs, the very little amount of yolk present is uniformly distributed throughout the ooplasm (for example, echinoderms, Amphioxus, mammals). This condition is usually observed in eggs with very little amount of yolk.
- **Centrolecithal Egg**: These eggs are relatively large in size, elongate and have a very great amount of yolk. The nucleus lies at the geometric centre of the yolk mass, surrounded by a small amount of cytoplasm. A thin cytoplasmic layer covers the surface of the yolk. Fine strands of cytoplasm extend from the peripheral layer to the zone occupied by the nucleus. Such as eggs of arthropods and some coelenterates.
- **Telolecithal Egg**: Eggs containing moderate or large quantity of yolk, where the distribution of the yolk is not uniform and is concentrated more towards the vegetal pole. Such a type of egg is called as telolecithal egg.

Telolecithal eggs may further be classified into three types:

- **Slightly Telolecithal**: These types of eggs contain only a small quantity of yolk; distributed unevenly where vegetal pole has the highest concentration and the animal pole has the lower (for example, eggs of fishes).
- **Moderately Telolecithal**: These types of eggs contain a moderate quantity of yolk which is distributed unevenly. Due to high concentration of yolk in the vegetal hemisphere, the nucleus is shifted more towards the animal hemisphere (for example, amphibian egg).
- **Extremely Telolecithal:** In this type of egg, due to the heavy deposition of yolk in the entire vegetal hemisphere, a major portion of the animal hemisphere is occupied by the yolk. Due to this extreme uneven distribution of yolk, the ooplasm and nucleus gets displaced towards the animal pole (for example, reptilian and avian eggs).

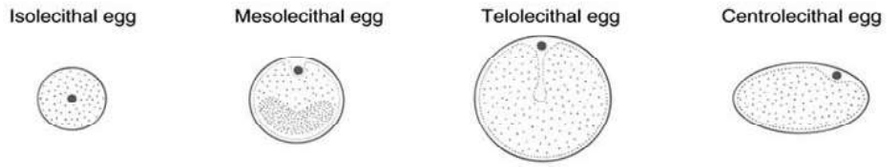

Fig. 3.6 *Types of Eggs in Chordates based on Distribution of Yolk*

On the Basis of Shell

Following are the classification of eggs on the basis of shell:

- **Cleidoic Egg**: These eggs contain a thick and hard outermost shell. This hard shell is permeable for gases. Yolk, salts and water is present in large amount in cleidoic eggs. Cleidoic egg is a terrestrial adaptation. For example: Birds and Reptiles, Prototheria mammal and insects.
- **Non Cleidoic Egg:** Egg membranes are soft in these eggs, for example, all viviparous animals and oviparous animals which lays eggs in water (Refer Figure 3.7).

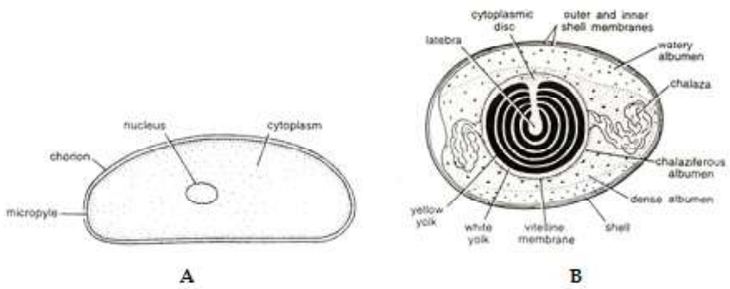

Fig. 3.7 *Cleidoic Eggs: Insect's Egg (A) and Hen's Egg (B).*

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On the Basis of Zonal Predetermination

Following are the classification of eggs on the basis of zonal predetermination:

- **Mosaic or Determinate Egg**: In certain eggs, every portion is predetermined with respect to its potentialities for further development. If a small portion of such an egg is removed, a defective embryo is formed this is because removal of a portion results in a permanent loss from the egg. The remaining portion of the egg cannot make compensatory development to make the lost part. Such an egg, in which the future developmental potentialities are predetermined in the form of a mosaic is called mosaic or determinate egg (for example, annelids, Molluscs and ascidians).
- **Regulative or Indeterminate Egg**: In vertebrates and most of the invertebrates, the developmental potentialities are not predetermined in the eggs. Removal of a small portion of the egg, or even one or two early blastomeres will not affect the normal development. This type of egg in which the future developmental potentialities are not predetermined is known as regulative or indeterminate egg.

3.3.4 Egg Polarity and Symmetry

Literal meaning of polarity is establishment of two opposite poles at both ends. Polarity is an essential feature of development in all organisms, from the 1-cell embryo to the establishment of tissue polarity through the whole organism. It is a characteristic of most cells in metazoan organisms and is associated with asymmetries in cell shape, protein content, organelle distribution and ultimately, cell function. Establishment and maintenance of polarity is of central importance in the process of oogenesis, particularly during oocyte maturation. Embryonic polarity in Xenopus and Drosophila are largely dependent upon the localization of mRNA determinants in the oocyte cortex. Two parts are said to be in symmetry when they appear identical after a flip, like reflection or mirror. The multicellular organism has different body parts which are formed from single cell. The distribution of the interior content of the first cell lay foundation for various developmental processes which results into the formation of different organs (Refer Figure 3.8).

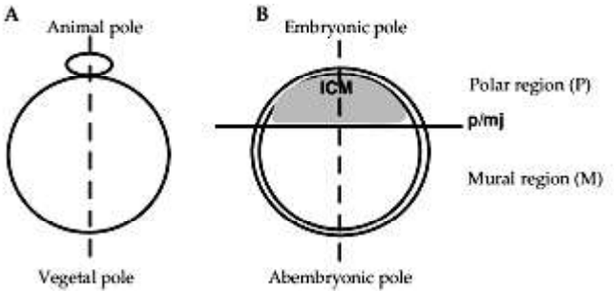

Fig.3.8 *Schematic Representation of Polarity and Symmetry in Egg*

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Origin of Polarity

Primary oocyte is an undifferentiated cell in the beginning of growth phase. When the growth and differentiation of oocyte is taking place inside the ovaries the polarity develops. Location of oocyte inside the ovary determines the future animal-vegetal axis of the ovum. Timing of attaining polarity is different with different species for example in mouse, polarity is first established in unfertilized oocytes which is characterized by the formation of metaphase spindle that is localized to the animal hemisphere of the oocyte. Chromosomal proximity leads to cortex reorganization in a Ran GTPase-dependent manner and induces actin, myosin 2, Par-3 and Par-6 accumulation above the spindle. Several studies showed that different cellular pathways such as Cdc42, Rac-GTPase and the Mos/MEK/MAP kinase pathway are also involved in the reorganization of the cortex above the spindle, as they regulate the translocation of the meiotic spindle to the oocyte cortex which accounts for the polarity of egg.

Factors Associated to Polarity of Egg

- **Uptake of Nutrients**: In determining the polarity of eggs, the uptake of nutrient plays a crucial role. For example in case of Molluscs, the end of oocyte which is attached to the wall of ovary will become the Vegetal pole of the egg. Therefore after this observation, it was concluded that the region of egg which is present in the close proximity of the ovary has more amount of yolk or in other words more yolk gets concentrated in that region. For example in case of Mammals and Amphibians they receive the nutrients from the follicular cells surrounding the ovaries but that doesn't turn whole of the egg into the vegetal pole.
- **Oxygen Consumption:** For the establishment of the vegetal pole most of the researchers believed that the end of oocyte which is present near the blood vessels gets more oxygen as compared to other end of ovum.
- **Electrical Field:** In 1934 Spek concluded the establishment of the electric field inside the egg which is responsible for the orientation and movement of molecules entering inside the egg at polar region in accordance to their electric charge.
- **Others:** There is large number of physical, chemical and environmental factors that helps in determining the egg polarity. Such as: electric current, light exposure, surrounding eggs, pH variation, temperature variation, site of sperm entry, nucleic acids etc.

Effect of Egg Polarity and Symmetry on Cleavage

Studies have shown that the symmetry inside the egg is distrupted by certain changes giving rise to egg polarity i.e. two distinct ends. An egg has two hemispheres called as animal pole and vegetal pole. Animal pole is more active, divides rapidly and participates in the formation of germ layers whereas vegetal pole contains more

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yolk content and divides slowly. Prior to fertilization, formation of animal-vegetal axis takes place. Sperm enters the egg from any point on the egg and defines it as dorso-ventral axis whereas the cells opposite to the entry of sperm forms dorsal portion. Polarity plays an important role in formation of embryonic structures. Asymmetric cell division is the main mechanism behind distinct fates of the cells. In case of *C. elegans,* 5 asymmetric divisions give rise to 6 founder cells namely AB, MS, E, C, D and P4 whose decedents further give rise to specific cell types as show below (Refer Figure 3.9):

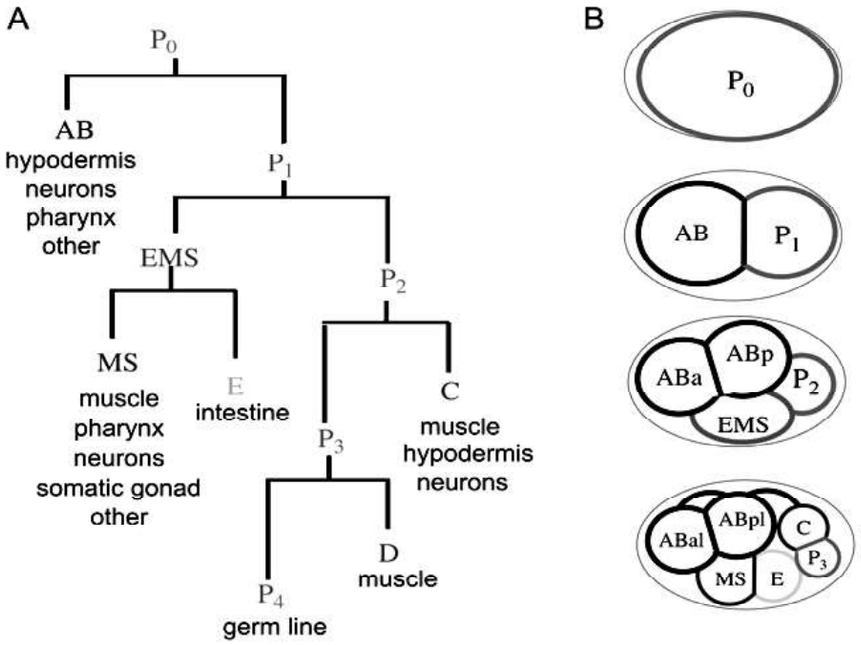

Fig. 3.9 *Polarity and Symmetry based Generation of Founder Cells in Early Embryo*

Thus, in animals, cell migration, asymmetric cell division, epithelial barrier function and morphogenesis takes place at the mercy of egg cell polarity.

Check Your Progress

1. Classify the eggs on the basis of shell.
2. Name the factors attributing towards the polarity of egg.
3. What kind of egg is present in Eutherian mammals?
4. What are the types of eggs on the basis of yolk distribution?
5. How many gonadotropic hormones are present in fish?

3.4 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

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1. The eggs on the basis of shell are: cleidoic and non-cleidoic eggs.
2. pH, Nutrients, Position of egg inside the ovary etc. are the factors attributing towards the polarity of egg.
3. Alecithal eggs is present in Eutherian mammals.
4. Isolecithal, centrolecithal, telolecithal and superficial are the types of eggs on the
5. One gonadotropic hormones is present in fish.

3.5 SUMMARY

- Maturation is the process in which prophase I arrested oocyte resume their meiosis. The term maturation is generally used to describe the completion of meiosis as two successive meiotic divisions occur in the animal oocyte, involving the nuclear and cytoplasmic changes.
- Ovum maturation or meiotic maturation and the ovulation forms the terminal stages of oogenesis, i.e., formation of female germ cells. Both nuclear and cytoplasmic changes occur during oocyte's final maturation, which have received relatively much more attention in other animal species than in reptiles and birds.
- Ovulation and maturation in vertebrates, including reptiles and birds are closely linked by common systemic factors which appear to correlate with the physiology of the ovary and the oocytes or shows that ovum maturation and ovulation in vertebrates are casually linked in some manner.
- In the frog *Rana pipiens*, duration of oogenesis is three years. The first two years of the oogenesis involves the gradual increase in the size of oocyte and in the last year of the oogenesis there is rapid accumulation of the yolk inside the egg which causes the swelling of the egg and help in attaining the big characteristic size.
- Ovulation occurs only after mating and is stimulated by the physical stirring of the cervix (mating). This physical stimulation of cervix trigger the Hypothalamus to release the Gonadotrophin Releasing Hormone (GnRH) which further stimulate anterior pituitary to release the gonadotrophin (FSH & LH).
- The gonadotrophin signals the ovary to reinitiate the process of meiosis followed by the process of ovulation, i.e., release of eggs. This mechanism of maturation of oocyte ensures the fertilization as process of ovulation is stimulated only by the mating.

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- Within 6 hours of progesterone stimulation, the nuclear membrane dissolves and this is known as Germinal Vesicle Breakdown (GVBD) as the nucleus in prophase is called as Germinal Vesicle.
- First meiotic division and the ovulation (i.e., release of ovum) take place from the ovaries, when the egg is released from the ovaries and is in the second meiotic metaphase stage.
- After the first meiotic division the oocytes again arrest its division at metaphase of second meiotic division, this block or arrest is caused by the CSF (Cytostatic Factor).
- Phosphorylation of Erp1 block the degradation of cyclin by Anaphase Promoting Complex and the arrest at the metaphase stage is broken by fertilization.
- In isolecithal eggs, the very little amount of yolk present is uniformly distributed throughout the ooplasm (for example, echinoderms, Amphioxus, mammals). This condition is usually observed in eggs with very little amount of yolk.
- Centrolecithal eggs are relatively large in size, elongate and have a very great amount of yolk. The nucleus lies at the geometric centre of the yolk mass, surrounded by a small amount of cytoplasm.
- A thin cytoplasmic layer covers the surface of the yolk. Fine strands of cytoplasm extend from the peripheral layer to the zone occupied by the nucleus. Such as eggs of arthropods and some coelenterates.
- Ovulation occurs only after mating and is stimulated by the physical stirring of the cervix (mating).
- Polarity is an essential feature of development in all organisms, from the 1-cell embryo to the establishment of tissue polarity through the whole organism. It is a characteristic of most cells in metazoan organisms and is associated with asymmetries in cell shape, protein content, organelle distribution and ultimately, cell function.
- Establishment and maintenance of polarity is of central importance in the process of oogenesis, particularly during oocyte maturation.
- Telolecithal Egg contain moderate or large quantity of yolk, where the distribution of the yolk is not uniform and is concentrated more towards the vegetal pole. Such a type of egg is called as telolecithal egg.
- Slightly Telolecithal eggs contain only a small quantity of yolk; distributed unevenly where vegetal pole has the highest concentration and the animal pole has the lower (for example, eggs of fishes).
- Primary oocyte is an undifferentiated cell in the beginning of growth phase. When the growth and differentiation of oocyte is taking place inside the ovaries the polarity develops. Location of oocyte inside the ovary determines the future animal-vegetal axis of the ovum.

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- Chromosomal proximity leads to cortex reorganization in a Ran GTPase-dependent manner and induces actin, myosin 2, Par-3 and Par-6 accumulation above the spindle.
- Several studies showed that different cellular pathways such as Cdc42, Rac-GTPase and the Mos/MEK/MAP kinase pathway are also involved in the reorganization of the cortex above the spindle, as they regulate the translocation of the meiotic spindle to the oocyte cortex which accounts for the polarity of egg.
- Oxygen Consumption for the establishment of the vegetal pole most of the researchers believed that the end of oocyte which is present near the blood vessels gets more oxygen as compared to other end of ovum.
- Electrical field in 1934 Spek concluded the establishment of the electric field inside the egg which is responsible for the orientation and movement of molecules entering inside the egg at polar region in accordance to their electric charge.
- An egg has two hemispheres called as animal pole and vegetal pole. Animal pole is more active, divides rapidly and participates in the formation of germ layers whereas vegetal pole contains more yolk content and divides slowly.
- In animals, cell migration, asymmetric cell division, epithelial barrier function and morphogenesis takes place at the mercy of egg cell polarity.

3.6 KEY WORDS

- **Maturation:** The process in which prophase I arrested oocyte resume their meiosis is called as maturation.
- **Estrus:** periodic ovulation pattern that is ovulation occurs only at specific period in a year. This kind of ovulation is called as Estrus /heat period/ oestrus
- **Telolecithal egg**: Eggs containing moderate or large quantity of yolk, where the distribution of the yolk is not uniform and is concentrated more towards the vegetal pole. Such a type of egg is called as telolecithal egg.
- **Centrolecithal egg**: The eggs that are relatively large in size, elongate and have a very great amount of yolk.
- **Isolecithal or homolecithal egg**: In isolecithal eggs, the very little amount of yolk present is uniformly distributed throughout the ooplasm.
- **Cleidoic egg**: The eggs having a thick and hard outermost shell. This hard shell is permeable for gases.
- **Non-cleidoic egg:** The eggs having a soft membranes, for example, all viviparous animals and oviparous animals which lays eggs in water.

3.7 SELF ASSESSMENT QUESTIONS AND EXERCISES

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Short Answer Questions

1. Write in short about maturation of egg.
2. Give a short note on maturation in frog.
3. How is mechanism of maturation carried out?
4. Give a brief note on resumption of Meiosis-II
5. What are the various classification of eggs that are done?
6. Give the classification of eggs on the basis of shell.
7. What is egg polarity and symmetry?

Long Answer Questions

1. How the egg attains animal and vegetal pole, explain?
2. Describe how egg maturity takes place in amphibian?
3. Give a detailed account on the classification of different types of eggs.
4. Elaborate the process of maturation of eggs.
5. What is meant by the symmetry and polarity of the egg?

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UNIT 4 MECHANISM OF FERTILIZATION

NOTES

Structure

4.0 INTRODUCTION

Fertilization is a phenomenon in which mature male gamete penetrate the mature female gamete ovum and this penetration leads to the fusion of their pronuclei resulting in the formation of the diploid cellular structure called as Zygote.

The idea of fertilization was known to Leeuwenhoek and according to him an ovum can develop into an animal only on its impregnation with sperm. In 1854, George Newport describes the entry of sperm into frog's egg. In 1875, Oscar Hertwig describe the union of nuclei of both the gametes, i.e., sperm and ovum during fertilization in Sea urchin. Fertilization is defined as the process of union of two gametes, eggs and sperm. When mammalian eggs and sperm come into contact in the female oviduct, a series of steps is set in motion that can lead to fertilization and ultimately to development of new individuals. Eggs are non-motile, surrounded by protective egg coverings. These serve to recognize the sperm specifically and prevent fertilization by more than one sperm (polyspermy).

The egg and the sperm are optimized in opposite ways for the propagation of the genes they carry. The egg is non-motile and aids the survival of the maternal genes by providing large stocks of raw materials for growth and development, together with an effective protective wrapping. The sperm, by contrast, is optimized to propagate the paternal genes by exploiting this maternal investment; it is usually highly motile and streamlined for speed and efficiency in the task of fertilization. Competition between sperm is fierce and the vast majorities fail in their mission. Out of the billions of sperm released during the reproductive life of a human male, only a few ever manage to fertilize an egg.

In this unit, you will study about fertilization and its type, mechanism of fertilization, monospermy and polyspermy, activation and metabolism of egg, etc. in detail.

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4.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand the fertilization and its type
- Explain the mechanism of fertilization
- Discuss the monospermy and polyspermy
- Describe the activation and metabolism of egg

4.2 FERTILIZATION: GENERAL ACCOUNT

The idea of fertilization was known to Leeuwenhoek and according to him an ovum can develop into an animal only on its impregnation with sperm. In 1854, George Newport describes the entry of sperm into frog's egg. In 1875, Oscar Hertwig describe the union of nuclei of both the gametes, i.e., sperm and ovum during fertilization in Sea urchin. Fertilization is defined as the process of union of two gametes, eggs and sperm. When mammalian eggs and sperm come into contact in the female oviduct, a series of steps is set in motion that can lead to fertilization and ultimately to development of new individuals. Eggs are non-motile, surrounded by protective egg coverings. These serve to recognize the sperm specifically and prevent fertilization by more than one sperm (polyspermy). The mammalian egg has zona pellucida layer around the plasma membrane beneath which cortical granules are present. The zona pellucida layer makes the egg impenetrable to more than one sperm. Sperms are highly motile cells consisting of nucleus and mitochondria to provide energy source and a flagellum for movement. The anterior end of the sperm is highly specialized which aids in penetration of the egg. Sperms are typically designed to activate the egg and to deliver their nuclei into the egg cytoplasm via seawater in marine forms, fresh water in fresh water forms and body fluid in viviparous animals. To increase the probability of fertilization, the number of sperms must exceed the number of eggs. Moreover the lifespan of gametes is limited; therefore fertilization must take place within a short duration of time. Eggs that are shed in water like that of most invertebrates, fishes and amphibians, have shorter life.

The egg and the sperm are optimized in opposite ways for the propagation of the genes they carry. The egg is non-motile and aids the survival of the maternal genes by providing large stocks of raw materials for growth and development, together with an effective protective wrapping. The sperm, by contrast, is optimized to propagate the paternal genes by exploiting this maternal investment; it is usually

highly motile and streamlined for speed and efficiency in the task of fertilization. Competition between sperm is fierce and the vast majorities fail in their mission. Out of the billions of sperm released during the reproductive life of a human male, only a few ever manage to fertilize an egg.

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4.2.1 Fertilization and its Types

Fertilization is a phenomenon in which mature male gamete peneteratrate the mature female gamete ovum and this penetration leads to the fusion of their pronuclei resulting in the formation of the diploid cellular structure called as Zygote. Fertilization is the second most important event in the process of sexual reproduction. There are four stages of fertilization:

- **Preparation**: It includes capacitation and acrosome reaction. The acrosomal vesicle fusion is the membrane fusion event of this stage.
- **Binding**: It is species-specific interaction of gametes.
- **Fusion:** Merging of sperm and egg plasma membranes is the membrane fusion event of this stage.
- **Activation**: It comprises cortical reaction (fusion of cortical vesicles with the egg plasma membrane) and pronuclear fusion.

The process of fertilization either taking place in surrounding medium outside of body or in side of body. It is a complicated process of sexual reproduction involves two gametes:

- Egg
- Sperm

Eggs: Eggs are large (~100µm), symmetrical and non-motile cells. Human eggs are arrested in metaphase of the second meiotic division and complete meiosis only upon fertilization. Their surface is covered by microvilli. Eggs are surrounded by zona pellucida which is a glycoprotein coat composed of three glycoproteins (ZPGP I-III). All three of the glycoproteins contain O- and N-linked oligosaccharides. The zona pellucida is not an osmotic barrier (in fact, even virus are capable of penetrating it), however it is a barrier to the sperm. The zona pellucida is the species specific barrier to fertilization as shown by the hamster experiment. Human sperm are incapable of fertilizing intact hamster eggs, but can fertilize hamster eggs stripped of their zona pellucida. This is used clinically to assess the fertilizing capacity of sperm (Refer Figure 4.1).

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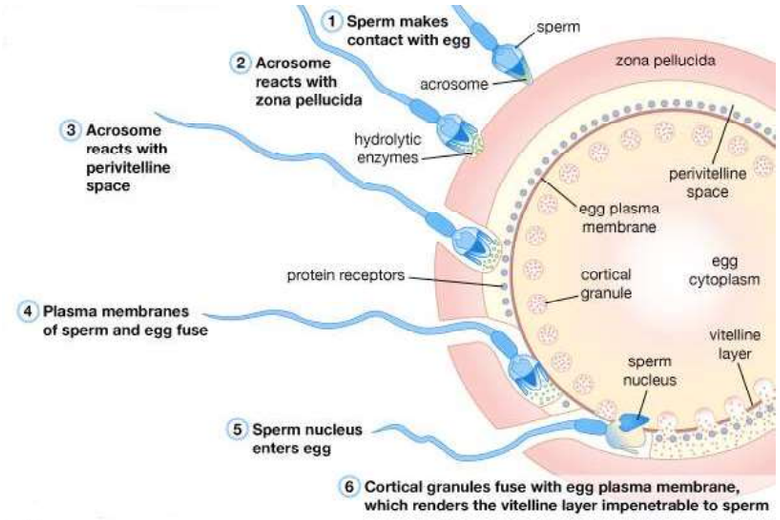

Fig. 4.1 *Diagrammatic Presentation of Fertilization*

Sperms: Sperms are small, asymmetrical and motile cells. They have components like:

- Acrosome
- Head
- Middle piece
- Tail

Acrosome: The acrosome is a lysosomal-like compartment derived from the Golgi. It has a low pH and contains soluble hydrolases (serine protease acrosin). In cross-section through the head of a sperm, one would cross four membranes in traversing from the plasma membranes to the nuclear membrane. During the acrosome reaction, fusion of the outer acrosomal membrane with the plasma membrane releases the contents of the acrosome and exposes the inner acrosomal membrane as the functional outer boundary of the sperm head.

Head: It contains the spermatic haploid nucleus. Overlaying the head is a membrane bound vesicle, the acrosome. Sperm do not possess any organelles associated with protein synthesis (Golgi body, RER or lysosomes). The sperm plasma membrane is also highly differentiated and contains proteins localized in distinct regions. One of these, termed PH-30 or fertilin, is localized in the equatorial region of the sperm and is involved in sperm-egg plasma membrane fusion.

Middle Piece: At the proximal portion of the tail. Mid piece contains a sheath of mitochondria, which produce the ATP necessary for the beating of the tail.

Tail: Also referred to as the principal piece. The tail contains the flagellar apparatus, which is composed of '9 + 2' microtubules and accessory structures. The sliding of the microtubule is powered by the protein dynein. (Gibbons' movie of sliding microtubules)

Types of Fertilization

The fertilization process in animals can occur either internally or externally, a difference which is largely determined by the method of birth.

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Internal Fertilization: Animals which use viviparous and ovoviviparous reproduction (embryos develop within the animal's body) and oviparous animals which lay hard shelled eggs, use internal fertilization. Internal fertilization involves the union of sperm and eggs within the body of the (usually female) parent. For internal fertilization to occur, the male must implant his sperm into the female reproductive tracts. Implantation can be achieved by either: Copulation, in which sperm transfer is performed by insertion of the penis or other male intromittent organ and ejaculation into the vagina, or cloaca,: or by a cloacal kiss, in which two birds press their cloacae together and sperm transfer takes place. Some animals, such as mollusks, arachnids, salamanders and certain insects, transfer a spermatophore, a bundle or capsule containing sperm, which is stored within the cloaca until oviposition takes place.

External Fertilization: Animals which are oviparous produce eggs which are lacking, or have thin egg membranes and reproduce by external fertilization. External fertilization is a reproductive strategy involving the joining of gametes outside of the body, either in a spawning event, where gametes from both sexes are rapidly released into an aquatic environment, or may occur when eggs are laid by a female on a substrate and are subsequently fertilized by a male. External fertilization holds certain benefits, such as reducing the chance of contracting sexually transmitted diseases, protection from violent behavior between organisms and increasing the genetic variation within a population.

4.2.2 Mechanism of Fertilization

The fertilization is a complicated four steps process as discussed below (Refer Figure 4.2):

Step-I Preparation of Sperm

Ejaculated sperms are not ready to fertilize an egg when they enter the vagina. In response to the dilution of semen in the vagina, they undergo several changes, which are collectively known as capacitation that includes:

- Intracellular Ca^{++} levels increases.
- Spermatic motility is activated and tail changes the beating frequency.
- Sperm cell surface antigens are lost. The loss of these proteins renders the sperm more receptive for binding to the egg.

Step-II Sperm-Egg Binding

The process of sperm-egg binding was first studied and understood in invertebrates. In sea urchins, the sperm head binds directly to the egg's outer surface and this triggers the acrosome reaction. The acrosomal contents are released and there is

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a balanced Na^+ influx and H^+ efflux, causing an increase in pH. The increased pH triggers the dissociation of the profilactin complex (actin and profilin) and the released actin monomers polymerize to form a filament called the acrosomal process. This acrosomal process penetrates the egg coatings to allow fusion of the sperm and egg plasma membranes. In sea urchins then, the sperm literally skewers the egg. In humans the process of sperm-egg binding is not so simple. The complicating factor is the thick zona pellucida, which keeps sperm from binding close to the egg membrane.

- **Sperm Receptor on Egg**: Dr. Paul Wassarman used a competition assay to isolate and identify the factor in the zona pellucida that was involved in sperm egg binding. He incubated sperm with Zona Pellucida Glycoproteins (ZPGPs) which was isolated from unfertilized and fertilized eggs. He found that sperm pre-incubated with ZPGPs from unfertilized eggs were not able to fertilize eggs. When he pre-incubated sperm with ZPGPs isolated from fertilized eggs, known not to bind sperm, the sperm could still fertilize eggs. This showed that the isolated ZPGPs from unfertilized eggs contain a receptor for the sperm and that receptor is modified after fertilization.

Dr. Barry Shur was studying a Golgi enzyme known as galactosyl transferase which catalyzes the addition of galactosyl residues from a donor nucleotide sugar, UDP-galactose, to the terminal end of O-linked oligosaccharides. As in all enzymatic reactions, there are two stages in catalysis:

- The enzyme binds the substrates (in this case UDP-gal and O-linked oligosaccharide, and
- The enzyme catalyzes the reaction and releases the products (in this case, UDP and the modified O-linked oligosaccharide with galacosyl residues on its ends).

It is important to understand that if one of the substrates is not present, the enzyme may be able to bind the available substrate, but will not be able to catalyze the reactions. This is important in sperm binding. Dr. Shur found that sperm, which have no Golgi apparatus, have galactosyl transferase on the surface of their plasma membrane. When sperms are ejaculated, they have oligosaccharides bound to the galactosyl transferase. During capacitation, these coating glycoproteins are removed, allowing the galactosyl transferase, to bind to other carbohydrates it may encounter, such as those attached to ZPGP III. However, there is no high energy UDP-galactose in the extracellular fluid surrounding the egg so catalysis does not occur and the sperm remains tightly bound to the egg zona pellucida. Many studies support a role of galactosyl transferase as a sperm protein involved in sperm-egg binding; however, other proteins may also be involved. A recent genetic knockout of galactosyl transferase in mice yielded mice that were completely fertile and showed normal sperm-egg binding.

Acrosome Reaction: As a result of irreversible binding of the sperm to the egg, the zona pellucida triggers the acrosome reaction. The outer plasma membrane

of the acrosome fuses at multiple sites with the plasma membrane and the contents of the acrosome are released. Two of the important components are acrosin, a serine protease and N-acetylglucoaminidase. Acrosin bores a hole in the zona pellucida so that the sperm can reach the egg itself. N acetylglucoaminidase hydrolyzes the O-linked oligosaccharides in ZPGP III to allow the sperm to detach. As a result of the membrane fusion, a new surface is exposed on the sperm (the inner acrosomal membrane) and this is thought to contain new binding sites for ZPGP II.

Step-III Sperm-Egg Fusion

For many years the process by which the plasma membrane of the sperm and egg fused, was a black box. Recent studies by Drs. Judith White, Diana Miles, and Paul Primakoff and their colleagues, have now shed light on this process. Miles and Primakoff made an antibody to PH-30, a heterodimeric sperm membrane protein comprised of á and â subunits and showed that this antibody blocked fertilization but did not block binding of sperm to eggs stripped of their zona pellucida. This suggested that PH-30 was involved in sperm and egg fusion and it was given the name Fertilin.

Cloning and sequencing of Fertilin revealed that á subunit had a hydrophobic domain that resembled with those on viral proteins that are known to be involved in membrane fusion. The â-subunit had a disintegrin domain. Disintegrins were first discovered in snake venom and act as competing ligands for integrins (for example, snake venom disintegrins, block platelet aggregation mediated by integrins). Fertilin was one of the first proteins of a family known as ADAMs proteins (for A Disintegrin and Metalloprotease containing protein) that are involved in cell-cell recognition and cell fusion events. Although the mechanism for how fertilin causes sperm-egg membrane fusion is not known, studies have supported its role in membrane fusion. For example, a peptide corresponding to the viral fusion peptide of á-fertilin is capable of fusing model membrane vesicles and the disintegrin domain of â-fertilin will block sperm-egg fusion. The egg integrin involved in sperm-egg fusion (the receptor for the â-subunit disintegrin) is known to be á6-â1. Once the sperm fuse with egg, the beating of tail stops immediately. The sperm instead is drawn into the egg by elongation and fusion of the egg's microvilli. As a result, the sperm nucleus and other organelles are incorporated into the egg cytoplasm. The sperm nucleus undergoes a series of changes, including chromatin decondensation and formation of a new nuclear envelope, to form a male pronucleus. The male pronucleus uses microtubules to migrate to the center of the cell, where it fuses with the female pronucleus to reconstitute a diploid nucleus. Other sperm organelles (for example, mitochondria) persist during early cleavage stages of the embryo and it is conjectured that they may play a role in development.

Step- IV Activation Response of Egg

The immediate events after fertilization include the egg's effort to prevent polyspermy. Polyspermy refers to the fertilization of the egg by more than one sperm, resulting

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in zygotes with greater than a diploid amount of DNA. This causes early embryonic defects and arrest of development. After sperm-egg fusion, the egg mounts the cortical reaction to prevent polyspermy. In all eggs, residing just under the plasma membrane there are membrane bound vesicles known as cortical granules. When a single sperm penetrates the egg, the cortical granules adjacent to the site are triggered to fuse with the plasma membrane, exocytosing their contents into the perivitelline space (the space between the plasma membrane and the zona pellucida). The cortical reaction is propagated over the surface of the egg by a wave of Ca^{++}. This was shown by the aequorin experiment in which the photoprotein aequorin phosphoresced in a wave from the site of sperm penetration of the egg. As a result of the cortical reaction, two important enzymes are released into the perivitelline space:

- **Ovoperoxidase:** In sea urchins, ovoperoxidase catalyzes the crosslinking of tyrosine residues in the extracellular matrix. This makes the extracellular matrix tough and insoluble (analogous to the tanning of leather) and a physical barrier is formed which prevents other sperm from fertilizing the egg. In mammals, ovoperoxidase does not catalyze tyrosine cross-linking to the point of insolubility. In mammals, its major effect is thought to be as a spermicial agent.

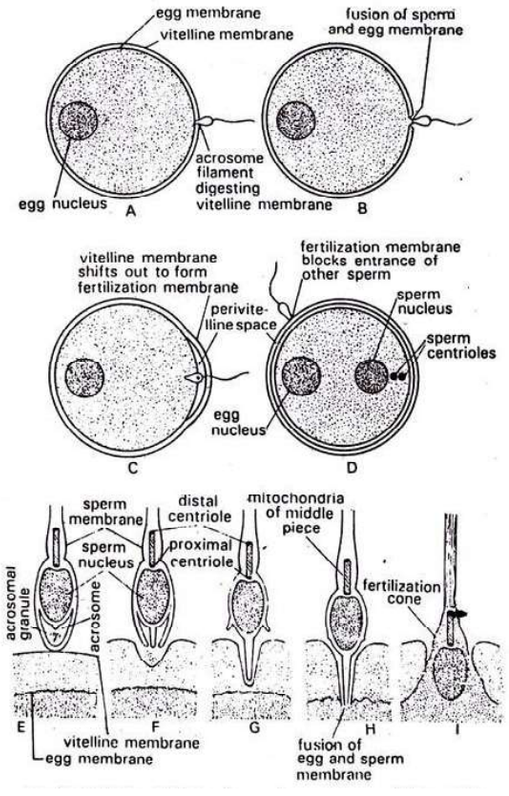

Fig.4.2 *Schematic Representation of Fertilization*

- **Hydrolase:** Remember Wassarman's result showing that zona pellucida from fertilized eggs was incapable of blocking fertilization? Another cortical granule that is released is a specific hydrolase, which degrades O-linked oligosaccharides on ZPGP III. This renders the Zona Pellucida incapable of binding additional sperm, thus preventing polyspermy. Activation of the egg also includes the initiation of development of the new zygote. Protein synthesis and other metabolic processes are upregulated to provide for the developing embryo.

4.2.3 Species Specific Fertilization by Fertilizin-Antifertilizin Reaction

One of the characteristic feature of fertilization is species specificity, i.e., spermatozoa of one species will only fertilize the ovum of that particular species only. Species specificity is of greatly biological importance as it helpful in maintaining the individuality of that species. This is achieved due to presence of specific chemical substances that are present on the surface of both the gametes. The Fertilizin secreted by the ovum and Anti-fertilizin secreted by sperm for the specific interaction which is essential for fertilization.

Fertilizin is a sperm –agglutinating agent produced by an ovum and plays an important role in the preliminaries of fertilization. Chemically it is glycoproteinaceous in nature composed of carbohydrates (Glucose+ Fructose+ Galactose) and amino acids. Specific interaction of fertilizin takes place with other chemical substance secreted by sperm known as Anti-fertilizin that causes the sperm to adhere to egg and penetrate. Anti-fertilizin is composed of acidic amino acids such as Glutamic acid and Aspartic acid. The interaction between both these chemical substances makes the spermatozoa stick to egg surface therefore this adhesion of spermatozoa to egg of the same species through a chemical recognition is called as Agglutination (Refer Figure 4.3).

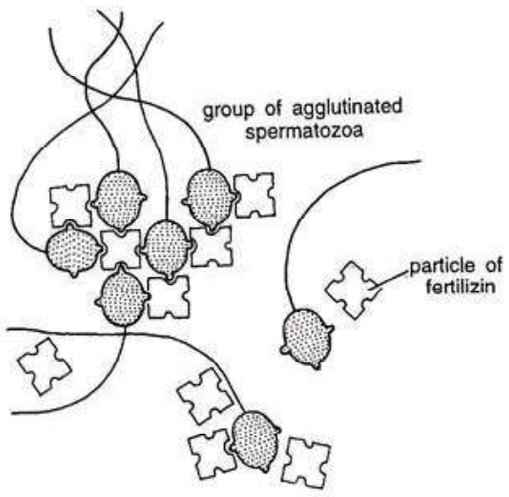

Fig. 4.3 *Agglutination of Sperm by Fertilizin and Anti-Fertilizing Reaction*

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Capacitation

After the ejaculation inside female genital tract mammalian sperm undergo a series of biochemical and physiological changes and are called as capacitation and these changes takes place in the female reproductive tract prior to the Acrosome Reaction (AR). Intracellular calcium plays an important role in bringing these changes in sperm. Ejaculated mammalian spermatozoa reside inside the female genital tract for several hours before acquiring the ability to fertilize the egg. In humans sperm must move out of the seminal plasma immediately after ejaculation and appear in the fallopian tube within minutes. These changes involve molecules absorbing on, or integrating into, the sperm plasma membrane during epididymal maturation. The removal or alteration of these molecules prepares the sperm toward successful binding to the egg and fertilization.

During mammalian fertilization, the capacitated spermatozoon penetrates the cumulus oophrous of the ovum, and then binds to the Zona Pellucida (ZP) with its plasma membrane intact. After binding to the egg ZP, the spermatozoon undergoes an exocytotic process called the Acrosome Reaction (AR). This event is required for fertilization, because it enables passage of the spermatozoon through the ZP and its subsequent fusion with the egg oolema.

The capacitation includes multiple physiological and biochemical modifications. The biochemical changes associated with the capacitation process include:

- An efflux of cholesterol from the plasma membrane leading to an increase in membrane fluidity permeability gets increased to bicarbonate and calcium ions
- Hyperpolarization of the plasma membrane
- Changes in protein phosphorylation and protein kinase activity
- Increase in bicarbonate (HCO_3^-) concentration and intracellular pH
- Ca^{2+} and cyclic Adenosine Monophosphate (cAMP) levels also gets increased

Generally the process lasts for 6 hours inside the female genital tract.

4.2.4 Monospermy and Polyspermy

As soon as sperm has entered the egg, the fusibility of the egg membrane, which was so necessary to get the sperm inside the egg, becomes a dangerous liability. In sea urchins, as in most animals studied, any sperm that enters the egg can provide a haploid nucleus and a centriole to the egg. In normal monospermy, in which only one sperm enters the egg, a haploid sperm nucleus and a haploid egg nucleus combine to form the diploid nucleus of the fertilized egg (zygote), thus restoring the chromosome number appropriate for the species. The centriole, which is provided by the sperm, will divide to form the two poles of the mitotic spindle during cleavage.

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The entrance of multiple sperm, polyspermy leads to disastrous consequences in most organisms. In the sea urchin, fertilization by two sperm results in a triploid nucleus, in which each chromosome is represented three times rather than twice. Worse, since each sperm's centriole divides to form the two poles of a mitotic apparatus, instead of a bipolar mitotic spindle separating the chromosomes into two cells, the triploid chromosomes may be divided into as many as four cells. Because there is no mechanism to ensure that each of the four cells receives the proper number and type of chromosomes, the chromosomes would be apportioned unequally. Some cells receive extra copies of certain chromosomes and other cells lack them. Theodor Boveri demonstrated in 1902 that such cells either die or develop abnormally.

Fast Block to Polyspermy

The fast block to polyspermy is achieved by changing the electric potential of the egg plasma membrane. This membrane provides a selective barrier between the egg cytoplasm and the outside environment and the ionic concentration of the egg differs greatly from that of its surroundings. This concentration difference is especially significant for sodium and potassium ions. Seawater has a particularly high sodium ion concentration, whereas the egg cytoplasm contains relatively little sodium. The reverse is the case with potassium ions. This condition is maintained by the plasma membrane, which steadfastly inhibits the entry of sodium ions into the oocyte and prevents potassium ions from leaking out into the environment. If we insert an electrode into an egg and place a second electrode outside it, we can measure the constant difference in charge across the egg plasma membrane. This resting membrane potential is generally about 70 mV, usually expressed as –70 mV because the inside of the cell is negatively charged with respect to the exterior. Within 1–3 seconds after the binding of the first sperm, the membrane potential shifts to a positive level, about +20 mV. This change is caused by a small influx of sodium ions into the egg. Although sperm can fuse with membranes having a resting potential of –70 mV, they cannot fuse with membranes having a positive resting potential, so no more sperm can fuse to the egg. It is not known whether the increased sodium permeability is due to the *binding* of the first sperm or to the *fusion* of the first sperm with the egg.

Slow Block to Polyspermy

The fast block to polyspermy is transient, since the membrane potential of the sea urchin egg remains positive for only about a minute. This brief potential shift is not sufficient to prevent polyspermy, which can still occur if the sperm bound to the vitelline envelope are not somehow removed. This removal is accomplished by the cortical granule reaction, a slower, mechanical block to polyspermy that becomes active about a minute after the first successful sperm-egg attachment.

Directly beneath the sea urchin egg plasma membrane is about 15,000 cortical granules, each cortical granule is about 1 ìm in diameter. Upon sperm

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entry, these cortical granules fuse with the egg plasma membrane and release their contents into the space between the plasma membrane and the fibrous mat of vitelline envelope proteins. Several proteins are released by this cortical granule exocytosis. The first are proteases. These enzymes dissolve the protein posts that connect the vitelline envelope proteins to the cell membrane and they clip off the binding receptor and any sperm attached to mucopolysaccharides (released by the cortical granules) produce an osmotic gradient that causes water to rush into the space between the plasma membrane and the vitelline envelope, causing the envelope to expand and become the fertilization envelope. A third protein released by the cortical granules, a peroxidase enzyme, hardens the fertilization envelope by cross-linking tyrosine residues on adjacent proteins. The fertilization envelope starts to form at the site of sperm entry and continues its expansion around the egg. As it forms, bound sperm are released from the envelope. This process starts about 20 seconds after sperm attachment and is completed by the end of the first minute of fertilization. Finally, a fourth cortical granule protein, hyalin, forms a coating around the egg. The egg extends elongated microvilli whose tips are attached to this hyaline layer. This layer provides support for the blastomeres during cleavage

4.2.5 Activation of Egg and Egg Metabolism

In Sea urchin the activation of egg and egg metabolism involves two mechanisms:

- Early Responses
- Late Responses

Early Responses: The activation of all eggs depends on an increase in the concentration of free calcium ions within the egg. Such an increase can occur in two ways either calcium ions can enter the egg from outside, or calcium ions can be released from the endoplasmic reticulum within the egg. Both processes vary in different species. In snails and worms, large amount of calcium probably enters the egg from outside, while in fishes, frogs, sea urchins, and mammals, most of the calcium ions probably come from the endoplasmic reticulum; however in both cases, a wave of calcium ions sweeps across the egg, beginning at the site of sperm-egg fusion. Presence of calcium ions is essential for activating the development of the embryo. Studies showed that the calcium-chelating chemical EGTA is injected into the sea urchin egg; there is no cortical granule reaction, no change in membrane resting potential and no reinitiation of cell division. Conversely, eggs can also get activated artificially in the absence of sperm by procedures that release free calcium into the oocyte.

Steinhardt and Epel in (1974) found that injection of micromolar amounts of the calcium ionophore A23187 into a sea urchin egg elicits most of the responses characteristic of a normally fertilized egg. The elevation of the fertilization envelope, a rise of intracellular pH, a burst of oxygen utilization and increases in protein and DNA synthesis are all generated in their proper order. In most of these cases, development ceases before the first mitosis because the egg is still haploid and

lacks the sperm centriole needed for the division. Calcium release activates a series of metabolic reactions. One of these is the activation of the enzyme NAD^+ kinase, which converts NAD^+ to $NADP^+$. This change may have important consequences for lipid metabolism, since $NADP^+$ (but not NAD^+) can be used as a coenzyme for lipid biosynthesis.

Thus, the conversion of NAD^+ to $NADP^+$ may be important in the construction of the many new cell membranes required during cleavage. Another effect of calcium release involves oxygen consumption. A burst of oxygen reduction (to hydrogen peroxide) is seen during fertilization and much of this 'respiratory burst' is used to crosslink the fertilization envelope. The enzyme responsible for this reduction of oxygen is also NADPH-dependent. Lastly, NADPH helps regenerate glutathione and ovothiols, which may be crucial for scavenging free radicals that could otherwise damage the DNA of the egg and early embryo.

Late Responses: Immediately after increase in the levels of calcium ion in sea urchin egg, its intracellular pH also increases and the rise in intracellular pH begins with a second influx of sodium ions, which causes a 1:1 exchange between sodium ions from the seawater and hydrogen ions from the egg. This loss of hydrogen ions is responsible for the rise in the intracellular pH. It is thought that the pH increase and the calcium ion elevation act together to stimulate new protein synthesis and DNA synthesis. If one experimentally elevates the pH of an unfertilized egg to a level similar to that of a fertilized egg, DNA synthesis and nuclear envelope breakdown ensue just as if the eggs were fertilized. The late responses of fertilization brought about by these ionic changes include the activation of DNA synthesis and protein synthesis. In sea urchins, a burst of protein synthesis usually occurs within several minutes after sperm entry. This protein synthesis does not depend on the synthesis of new messenger RNA; rather, it utilizes mRNAs already present in the oocyte cytoplasm. These messages include mRNAs encoding proteins such as histones, tubulins, actins, and morphogenetic factors that are utilized during early development. Such a burst of protein synthesis can be induced by artificially raising the pH of the cytoplasm using ammonium ions.

Check Your Progress

1. Name the glycoproteins present on the surface of Zona Pellucida.
2. How many cortical granules are present in the egg?
3. What ions are essential for the activation of egg metabolism?
4. Name the chemical substances used for maintaining the species-specificity.
5. What happens when the egg gets fertilized?

4.3 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

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1. ZP-I,ZP-II, and ZP-III glycoproteins is present on the surface of Zona Pellucida
2. 15000 granules are present in the egg.
3. Calcium ions are essential for the activation of egg metabolism.
4. Fertilizin and Antifertilizin the chemical substances used for maintaining the species-specificity.
5. Nuclear envelope degenerate and exchange of genetic material takes place when the egg gets fertilized.

4.4 SUMMARY

- The idea of fertilization was known to Leeuwenhoek and according to him an ovum can develop into an animal only on its impregnation with sperm. In 1854, George Newport describes the entry of sperm into frog's egg. In 1875, Oscar Hertwig describe the union of nuclei of both the gametes, i.e., sperm and ovum during fertilization in Sea urchin.
- Fertilization is defined as the process of union of two gametes, eggs and sperm. When mammalian eggs and sperm come into contact in the female oviduct, a series of steps is set in motion that can lead to fertilization and ultimately to development of new individuals.
- Eggs are non-motile, surrounded by protective egg coverings. These serve to recognize the sperm specifically and prevent fertilization by more than one sperm (polyspermy).
- The mammalian egg has zona pellucida layer around the plasma membrane beneath which cortical granules are present. The zona pellucida layer makes the egg impenetrable to more than one sperm.
- Sperms are highly motile cells consisting of nucleus and mitochondria to provide energy source and a flagellum for movement.
- The anterior end of the sperm is highly specialized which aids in penetration of the egg. Sperms are typically designed to activate the egg and to deliver their nuclei into the egg cytoplasm via seawater in marine forms, fresh water in fresh water forms and body fluid in viviparous animals.
- Eggs that are shed in water like that of most invertebrates, fishes and amphibians, have shorter life.

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- The egg and the sperm are optimized in opposite ways for the propagation of the genes they carry.
- Copulation, in which sperm transfer is performed by insertion of the penis or other male intromittent organ and ejaculation into the vagina, or cloaca,: or by a cloacal kiss, in which two birds press their cloacae together and sperm transfer takes place.
- Animals which are oviparous produce eggs which are lacking, or have thin egg membranes and reproduce by external fertilization.
- External fertilization is a reproductive strategy involving the joining of gametes outside of the body, either in a spawning event, where gametes from both sexes are rapidly released into an aquatic environment, or may occur when eggs are laid by a female on a substrate and are subsequently fertilized by a male.
- Acrosome reaction is a result of irreversible binding of the sperm to the egg, the zona pellucida triggers the acrosome reaction. The outer plasma membrane of the acrosome fuses at multiple sites with the plasma membrane and the contents of the acrosome are released.
- Ovoperoxidase in sea urchins, ovoperoxidase catalyzes the crosslinking of tyrosine residues in the extracellular matrix.
- Species specificity is of greatly biological importance as it helpful in maintaining the individuality of that species.
- Fertilizin is a sperm –agglutinating agent produced by an ovum and plays an important role in the preliminaries of fertilization.
- Anti-fertilizin is composed of acidic amino acids such as Glutamic acid and Aspartic acid.
- Ejaculated mammalian spermatozoa reside inside the female genital tract for several hours before acquiring the ability to fertilize the egg.
- During mammalian fertilization, the capacitated spermatozoon penetrates the cumulus oophrous of the ovum, and then binds to the Zona Pellucida (ZP) with its plasma membrane intact.
- The centriole, which is provided by the sperm, will divide to form the two poles of the mitotic spindle during cleavage.
- The fast block to polyspermy is achieved by changing the electric potential of the egg plasma membrane. This membrane provides a selective barrier between the egg cytoplasm and the outside environment and the ionic concentration of the egg differs greatly from that of its surroundings.
- In sea urchins, as in most animals studied, any sperm that enters the egg can provide a haploid nucleus and a centriole to the egg.

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4.5 KEY WORDS

- **Fertilisation:** Fertilisation or fertilization, also known as generative fertilisation, insemination, pollination, fecundation, syngamy and impregnation, is the fusion of gametes to initiate the development of a new individual organism or offspring.
- **Sperm:** Sperm is the male reproductive cell and is derived from the Greek word sperma.
- **Acrosome:** The acrosome is an organelle that develops over the anterior half of the head in the spermatozoa (sperm cells) of many animals including humans
- **Ejaculation:** Ejaculation is the discharge of semen from the male reproductory tract, usually accompanied by orgasm..
- **Capacitation:** Capacitation is the penultimate step in the maturation of mammalian spermatozoa and is required to render them competent to fertilize an oocyte.

4.6 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short Answer Questions

1. Write short note on fertilization.
2. What are the various ways of fertilisation?
3. Brief a note on sperm and its parts.
4. Distinguish between internal and external fertilisation.
5. Write short note on sperm receptor on egg.
6. What is capacitation?

Long Answer Questions

1. Describe the major changes taking place in the egg when sperm enters the reproductive track.
2. Elaborate different types of fertilization with suitable examples.
3. Describe activation of egg and egg metabolism in detail.
4. Explain different stages of fertilization.
5. Give an account of mechanism fertilization in different animals.
6. Write short notes on following:
    - Monospermy and polyspermy
    - Capacitation
    - External and internal fertilization

4.7 FURTHER READINGS

NOTES

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BLOCK - II

CLEAVAGE AND GASTRULATION

UNIT 5 CLEAVAGE AND CHEMODIFFERENTIATION

Structure

5.0 INTRODUCTION

Just after the fertilization, the most crucial event in the lifetime of an organism is the process of sequential mitotic divisions known as 'cleavage'. The zygote resulted from the fusion of male and female gamete starts dividing mitotically in which the enormous amount of egg cytoplasm is divided into number of cells known as blastomeres. Cleavage is also known as segmentation and thus this process is defined as series of successive and synchronic mitotic divisions of zygote forming blastomeres which are quintessential for the development of zygote into an offspring. In utmost organisms except mammals, initially the rate of cell division as well as the positioning of blastomeres with respect to each other is entirely controlled by the factors such as proteins and mRNA stored in oocyte's cytoplasm. With the progression of cell divisions the rate of divisions and the positioning of blastomeres come under the control of newly formed zygotic genome. In case of invertebrates, cleavage occurs very rapidly for generating numerous cells to restore somatic ratio, i.e., ratio of nuclear volume to cytoplasmic volume and the dividing zygote does this by terminating the gap period between G1 and G2 (Growth Phases) of a cell cycle. For example, frog's egg, in just 43 hours divides into 37000 cells whereas; *Drosophila's* egg divides into 50000 cells in just 12 hours.

In this unit, you will study about cleavage, its types, patterns and importance. This unit will also teach you about chemodiffrentitaion and the factors governing it.

5.1 OBJECTIVES

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After going through this unit, you will be able to:

- Understand cleavage and laws of cleavage
- Know the patterns, types and importance of cleavage
- Learn about factors affecting cleavage and chemodiffrentitaion

5.2 CLEAVAGE

Process of cleavage was first observed by Swammerdam in 1738 in the egg of frog. First two cleavage planes in case of toad's egg were described by Spallanzani in 1780. The entire process of cleavage in frog's egg was studied by Prevost and Dumas in 1824. Cleavage is actually the result of two coordinated processes. The first of these cyclic processes is karyokinesis, the mitotic division of the nucleus. The mechanical agent of this division is the mitotic spindle, with its microtubules composed of tubulin (the same type of protein that makes up the sperm flagellum). The second process is cytokinesis, the division of the cytoplasm. The mechanical agent of cytokinesis is a contractile ring of microfilaments made of actin (the same type of protein that extends the egg microvilli and the sperm acrosomal process). The mitotic spindle and contractile ring are perpendicular to each other and the spindle is internal to the contractile ring. The contractile ring creates a cleavage furrow, which eventually bisects the plane of mitosis, thereby creating two genetically equivalent blastomeres.

The actin microfilaments are found in the cortex of the egg rather than in the central cytoplasm. Under the electron microscope, the ring of microfilaments can be seen forming a distinct cortical band 0.1μm wide. This contractile ring exists only during cleavage and extends 8–10μm into the center of the egg. It is responsible for exerting the force that splits the zygote into blastomeres; if it is disrupted, cytokinesis stops. Schroeder (1973) has proposed a model of cleavage wherein the contractile ring splits the egg like an 'intercellular purse-string,' tightening about the egg as cleavage continues. This tightening of the microfilamentous ring creates the cleavage furrow. Microtubules are also seen near the cleavage furrow (in addition to their role in creating the mitotic spindles), since they are needed to bring membrane material to the site of membrane addition. Although karyokinesis and cytokinesis are usually coordinated, they are sometimes separated by natural or experimental conditions. In insect eggs, karyokinesis occurs several times before cytokinesis takes place. Another way to produce this state is to treat embryos with the drug cytochalasin B. This drug inhibits the formation and organization of

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microfilaments in the contractile ring, thereby stopping cleavage without stopping karyokinesis (Schroeder 1972), (Refer Figure 5.1).

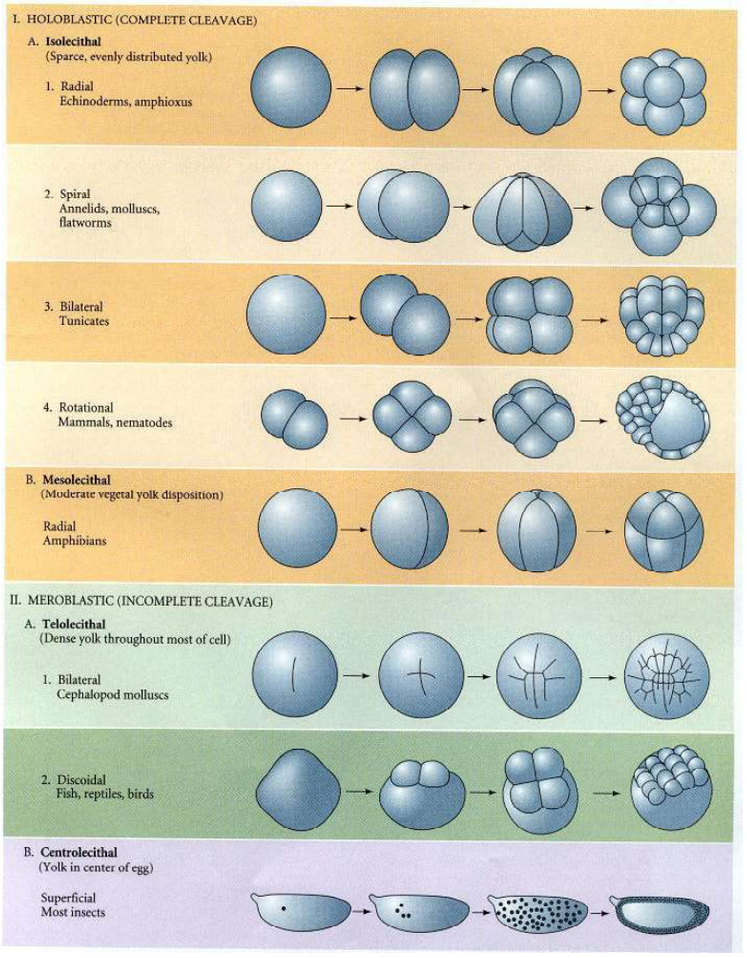

Fig. 5.1 *Comparative Account of Various Patterns of Cleavage in Different Types of Eggs*

Law of Cleavage

There are four major cleavage laws, known by the names of those who first emphasized their importance:

- **Pfluger's Law**: The spindle elongates in the direction of least resistance.
- **Balfour's Law**: The rate of cleavage tends to be governed by the inverse ratio of the amount of yolk present, in holoblastic cleavage. The yolk tends to impede division of both the nucleus and the cytoplasm.
- **Sack's Law**: Cells tend to divide into equal parts and each new plane of division tends to bisect the previous plane at right angles.
- **Hertwig's Law:** The nucleus and its spindle generally are found in the center of the active protoplasm and the axis of any division spindle lies in

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the longest axis of the protoplasmic mass. Divisions tend to cut the protoplasmic masses at right angles to their axes.

Planes of Cleavage

During early cleavage, distinct geometrical relationships exist between the blastomeres i.e., each plane of cell-division bears a definite relationship with each other. There are different plains of cleavage based on the types and groups of eggs:

- **Meridional Plane of Cleavage:** When a furrow bisects both the poles of the egg passing through the median axis or centre of egg it is called meridional plane of cleavage. The median axis runs between the centre of animal pole and vegetal pole.
- **Vertical Plane of Cleavage:** When a furrow passes in any direction (does not pass through the median axis) from the animal pole towards the opposite pole.
- **Equatorial Plane of Cleavage:** This type of cleavage plane divides the egg halfway between the animal and vegetal poles and the line of division runs at right angle to the median axis.
- **Latitudinal Plane of Cleavage:** This is almost similar to the equatorial plane of cleavage, but the furrow runs through the cytoplasm on either side of the equatorial plane.

Types of Cleavage

Considerable amount of reorganization occurs during the period of cleavage and the types of cleavage depend largely upon the cytoplasmic contents. Different types of cleavage encountered in different eggs are catalogued below (Refer Figure 5.2):

On the Basis of Amount of Distribution of Yolk

Yolk is a heterogenous chemical substance that retards the cleavage furrows which divide the cytoplasm. An enormous amount of yolk tends to displace the mitotic apparatus to an off centre position hence the amount and distribution of yolk greatly determines the different types of cleavage in different organisms.

i. **Holoblastic or Total Cleavage:** In this type of cleavage the zygote and blastomeres are divided completely by cleavage furrow or in other words when the cleavage furrows divide the entire egg. This kind of cleavage occurs in different types of ovum such as alecithal, homolecithal and little telolecithal. Further this kind of cleavage is of two types: equal holoblastic cleavage and unequal holoblastic cleavage, discussed below:
    - **Equal Holoblastic Cleavage :** When the cleavage furrow cuts the egg into two equal cells and result into the production of the nearly

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equal –sized blastomeres throughout the whole cleavage process because the mitotic apparatus is formed near the centre of the zygote or the blastomere. It may be radially, bilaterally, spirally or irregular in symmetry. Found in the alecithal egg of rabbit and microlecithal egg of sea squirt.

- **Unequal Holoblastic Cleavage:** This kind of cleavage results into the formation of two different types of blastomeres of unequal size. One is small sized blastomeres called as micromeres at the animal pole and large sized blastomeres called as macromeres at the vegetal pole. Found in telolecithal eggs of frog and bony fishes. In these eggs, the yolk displaces the mitotic apparatus into animal hemisphere and cleavage furrow is more prominent in the animal hemisphere as compared to vegetal hemisphere that's why numerous and smaller micromeres are found in this region.

ii. **Meroblastic Cleavage:** In this type of cleavage, mitotic divisions occur only in the metabolically active cytoplasm which remains confined to the animal or the peripheral region of the egg. However the yolk remains undivided and this kind of cleavage generally found in macrolecithal eggs or in other words, when segmentation takes place only in a small portion of the egg resulting in the formation of blastoderm, it is called meroblastic cleavage. Usually the blastoderm is present in the animal pole and the vegetal pole becomes laden with yolk which remains in anticleaved state, i.e., the plane of division doesnot reach the periphery of blastoderm or blastodisc. Further meroblastic cleavage is of two different types: discoidal cleavage and superficial cleavage:

- **Discoidal Cleavage:** This type of cleavage is the characteristic feature of (meiolecithal eggs) reptiles, birds and other egg laying mammals. A rapid mitotic division occurs only in the cytoplasmic disc present in the animal pole of the egg resulting in the formation of different layer of cells. Also, in such eggs there is displacement of the mitotic apparatus towards the cytoplasmic disc under influence of yolk.
- **Superficial Cleavage:** This type of cleavage is the characteristic feature of centrolecithal eggs of insects. Nucleus also known as the synkaryon is present in the central mass of the cytoplasm, called as 'energid' at the middle of the yolk mass and undergo many repeated mitotic divisions to form large number of daughter nuclei and all of these daughter nuclei migrates through the cytoplasmic connections towards the yolk free cytoplasm present at the peripheral region of the egg. This whole process makes an egg a syncytium. Later on the mitotic divisions takes place and form many multinucleated blastomeres arranged around the yolk. However only few nuclei remain inside central cytoplasm for the digestion of the yolk. Relatively thick region of

periplasm present at the posterior region of the pole called as the Pole Plasm get cleaved into the pole cells after receiving some energies.

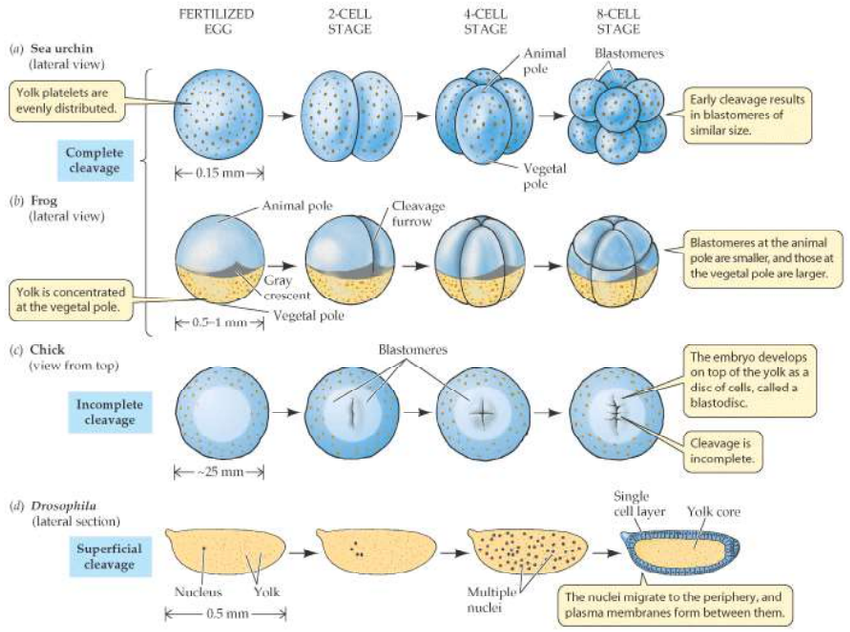

Fig. 5.2 *Types of Cleavage on the Basis of Yolk (Amount and Distribution)*

On the basis of Orientation of Mitotic Apparatus

There are various types of cleavage reported in different group of animals on the basis of mitotic orientation:

- **Radial Cleavage**: In this kind of cleavage mitotic apparatus is either parallel or perpendicular to the animal-vegetal axis of the egg and cleavage furrow appears in a manner that it lies radial to the primary axis of the egg. Radial cleavage mostly observed in 8- celled stage where the upper blastomeres lie directly above the lower four cells. Any line passing through the animal vegetal axis of the egg divides the egg into symmetrical halves. It is greatly observed in the sponges, coelenterates and echinoderms such as starfish.
- **Spiral Cleavage**: In this cleavage the mitotic apparatus is inclined with respect to animal-vegetal axis of the egg and the blastomeres arranged in the spiral manner around the axis. When the mitotic apparatus is inclined / tilted in clockwise direction then after third cleavage the upper micromeres is displaced obliquely to the right side of lower tier of micromere. This right handed or clockwise displacement of micromeres is called dextral spiral cleavage. In some species, the mitotic apparatus is inclined towards anti-clockwise direction than the micromeres are displaced obliquely to the left side of the macromeres. This left-handed or left side inclination of the macromeres is called as the sinistral spiral cleavage.

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- **Bilateral Cleavage:** In this type of cleavage the mitotic apparatus is present either perpendicular or parallel to the animal-vegetal axis of the egg and the resulting blastomeres lies in the radial manner about the primary egg axis as in case of radial cleavage. But in this case the right and left side of the embryo are distinguishable and only one plane can divide it into the similar halves. It occurs in case of molluscs, fishes, amphibians etc.
- **Rotational Cleavage**: In this kind of cleavage first two blastomeres rotates about at an angle of 90 degree with respect to each other before the commencement of second cleavage which occurs in two planes, i.e., meridional in one blastomere and equatorial in another. It occurs in the wide range of nematodes.

Types of Cleavage on the Basis of Potentiality of Blastomeres

There are basically two types of cleavage reported on the basis of future scope and potentiality of blastomeres:

- **Determinate Cleavage:** In Animals showing spiral cleavage, their resultant blastomere's fate is fixed right from the beginning of first division of the zygote. Consequenlty complete embryo will be formed only if all the blastomeres remain adhered together; if any of the blastomeres looses the contact with another then their fate deciding ability also gets lost.
- **Indeterminate Cleavage:** During this kind of cleavage the fate of blastomeres is not fixed; in other words they can form any part of embryo. Usually this kind of cleavage is found in Ascidians.

Effects of Yolk in Cleavage

The fertilized egg in most cases contains yolk, which are inert bodies. During division these bodies exert mechanical influences. In the egg of Amphioxus, the yolk is thin and remains uniformly distributed. Therefore the division is complete and early divisions occur at a very quicker rate. The amphibian egg contains yolk which is localized at the vegetal pole. Here division gets initiated from the animal pole and extends towards the vegetal pole, where the progress of cleavage slows down considerably. Consequently, the animal pole divides faster than the vegetal pole. The eggs of reptiles and birds are fully laden with large masses of yolk, thus restricting the cytoplasm and nucleus on the periphery as a circular disc on the animal pole. The lines of cleavage divide only the small animal pole region. Such effects of yolk on cleavage pattern influence the pattern of further development.

Mechanism of Cleavage

The incidence of cleavage provides unique opportunity to study the mechanism of cell division and specially the role of different cell organelles during division. Opinions differ regarding the accumulation of force for the initiation of cleavage and following factors are believed to be responsible for controlling the cleavages:

- Localized expansion of cortex

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- Increased stiffness of the cortical cytoplasm
- Increase of tangential force activity in the cortex
- Contractile nature of the regions near the cortex
- Formation of new cell membrane from the sub cortical cytoplasm.

Though the above mentioned factors are not clearly understood, it is evident that three structures present within the cell: Cortical layer, Spindle structures and Chromosomes play the important part. The energy which is required during the process is supplied by the metabolic activity of the developing egg. Besides the factors involved in segmentation, there are cleavage laws which govern the behaviour of the cells during cleavage. These are: Sach's rules (The blastomeres tend to divide into identical daughter cells and a cleavage furrow tends to cut the previous cell at right angles); Hertwig's laws (The position of nucleus is vital and it tends to lie at the centre of the protoplasmic content of the cell. The nucleus exerts influence on cleavage. The long axis of mitotic spindle usually coincides with the long axis of the protoplasmic content. During cleavage the long axis of the protoplasm has the tendency to cut transversely); and Balfour's law (The rate of cleavage is inversely proportional to the amount of yolk material present in the egg).

Chemical Changes During Cleavage

Significant chemical changes go on in the fertilized egg during cleavage. These changes are:

- **Increase of Nuclear Material:** During cleavage a steady increase in nuclear material (predominantly DNA) is observed. Cytoplasm of the egg is the source of such nuclear material. Cytoplasmic DNA contained in mitochondria and yolk platelets are available.
- **RNA Synthesis:** During cleavage messenger RNA (mRNA) and transfer RNA (tRNA) are synthesized during cleavage, especially in late stages.
- **Synthesis of Proteins:** Throughout the period of cleavage there is steady and spectacular increase in protein synthesis.

5.2.1 Cleavage in Frog

The egg of frog is telolecithal with a considerable amount of yolk localized towards the vegetal pole. The cleavage is holoblastic in nature, but differs considerably from that of *Amphioxus* because of larger quantity of yolk. The first cleavage plane is meridional which occurs at about 3-3½ hours after fertilization. But the time depends largely on extrinsic factors. The first cleavage starts at the animal pole and gradually travels towards the vegetal pole. Thus the egg is bisected along the poles and two blastomeres of equal size are produced. The second cleavage is almost meridional but oriented at right angles to the first cleavage plane (Refer Figure 5.3).

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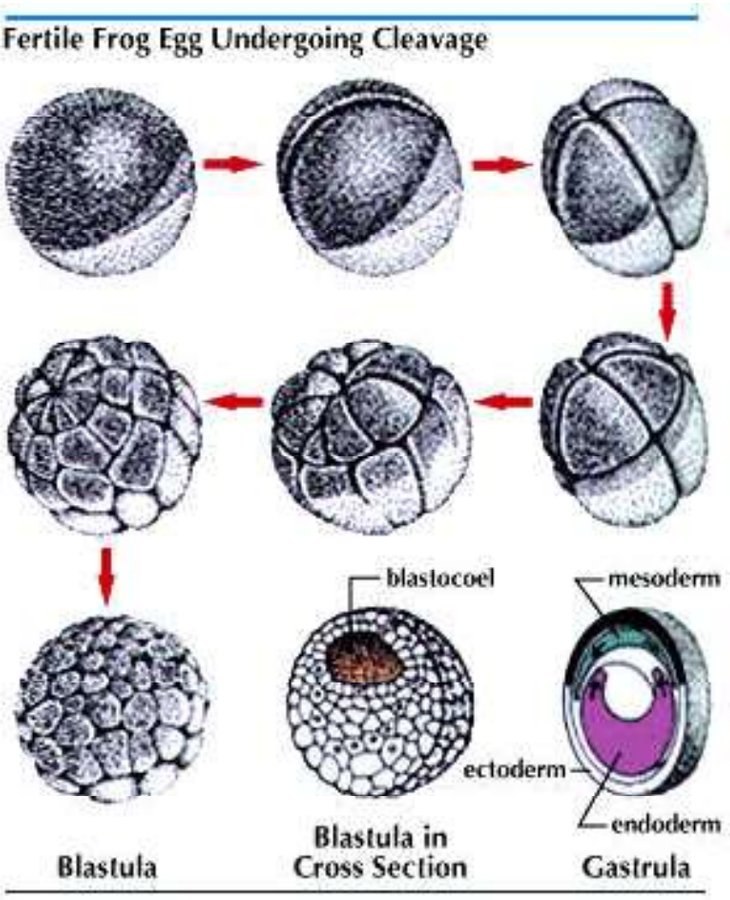

Fig. 5.3 *Schematic Diagram of Cleavage in Frog*

The four blastomeres thus produced are not qualitatively identical, because the grey crescent material is present in two of the four blastomeres. Each blastomere contains dark pigment at the animal pole and yellowish yolk towards the vegetal pole. The third cleavage is latitudinal and occurs at right angles to previous cleavage planes but passes slightly above the equator. The furrow produces eight unequal blastomeres, four micromeres in the animal hemisphere and four macromeres in the vegetal part. The fourth cleavage planes are meridional which involve the micromeres first and pass on slowly towards the yolk-laden macromeres of the vegetal pole. In *Amphioxus*, the cleavages occur in a synchronous fashion, while in frog considerable degree of irregularities (asynchronism) appears in later stages. But it is certain that the micromeres always continue to divide at a faster rate than the macromeres. At the eight-celled stage, a small space makes its appearance between the four micromeres. As development goes on, this space becomes conspicuous and forms the blastocoel. The floor of the blastocoel is formed of macromeres. The blastocoel (or segmentation cavity) is eccentrically located and becomes displaced towards the animal pole as development proceeds.

5.2.2 Cleavage in Chick

Typical meroblastic cleavage occurs in chick, where the segmentation activity is restricted only at the blastodisc or germinal disc. Thus the cleavage is incomplete. The first cleavage starts as a meridional furrow near the centre of the blastodisc at about 4½ hours after fertilization when the egg reaches the isthmus of oviduct. This furrow cuts across the blastodisc and passes towards the vegetal pole but does not reach the pole. The second cleavage is also meridional, but approximately at right angles to the first one. The third cleavage is vertical. The fourth cleavage is also vertical but the division is not synchronous. As a consequence eight central cells encircled by twelve marginal cells are produced. From this point onward the cleavage becomes irregular and a disc containing smaller cells appears. This disc remains firmly connected with the underlying yolk. Soon a cleft appears which separates the disc in the middle from the underlying yolk. The new cavity in between is known as sub-germinal space (Refer Figure 5.4).

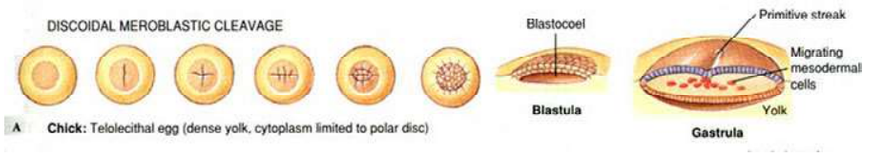

Fig. 5.4 *Schematic Diagram of Cleavage in Chick*

Thus at the end of segmentation, the disc contains many-layered small cells which are connected with the yolk only at the periphery. This disc is then termed as blastoderm, the cells of which still continue to divide. The peripheral part which lies in contact with yolk possesses granular cells called area apaca and the inner layer having clear portion is called area pellucida. At one end of area opacas, aggregation of cells takes place. This denotes the formation of future posterior side.

Significance of Cleavage in Embryogenesis

The cleavage phase of development and blastulation are extremely significant, because the blastoderm is morphologically elaborated in such a way that the important presumptive organ forming areas of the future embryo are segregated into definite districts of the blastoderm. Such orientation of the organ forming areas in the blastoderm permits an ordered movement of these areas during gastrulation to take up their fateful position. So the period of cleavage and blastulation is regarded as the phase of preparation for future differentiation. The cells which are produced at the end of segmentation resemble the zygote, but do they possess the same potentiality as the zygote itself?

Driesch (1891), in order to get an answer, separated the two blastomeres at the two-celled stage and found that both the blastomeres developed into complete

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embryos. His conclusion was that each blastomere has the full potentiality to be an entire embryo. But in 1900, Roux showed that if one of the blastomeres of the two-celled stage is killed, the remaining one produces 'half embryo'. He claimed that each cleavage results into the segregation of specialization in the blastomeres and this is irreversible. This experiment demonstrates that an organizing or controlling centre is elaborated to control the development process. The experiment of Spemann and others have shown that it is the grey crescent region which plays the vital role in the process of determination and the blastomeres which are formed due to segmentation are neither completely regulative nor irreversibly determined. It has been experimentally established that the grey crescent in the amphibian blastula transforms into the dorsal lip of the blastopore which acts as an instigator and controller of the gastrulation process.

5.3 CHEMODIFFERENTIATION

As the late blastula converses into the late gastrula, the presumptive neural plate ectodermal and epidermal ectodermal areas become changed physiologically, as a result they no longer are determined in a presumptive sense but have undergone changes which make them self-determining. This change is called determination and the biochemical change which effects this alteration is known as chemo-differentiation. As chemo-differentiation involves physiological changes, it restricts changes in potency upon many localized cellular areas. As a result various future organs and parts of organs have their respective fates rigidly and irrevocably determined at the end of gastrulation. Chemodifferentiation apparently occurs through inductive action. Chemical basis of differentiation or chemodifferentiation of any cell is based on the various differentiation or diversification products or enzymes which direct the synthesis of organic compounds that imparts the uniqueness to cell. According to Spiegelman 1948 differentiation is the product of unique enzyme patterns and according to Balinsky 1970 differentiation is the production of unique patterns.

Check Your Progress

1. Who discovered the process of cleavage?
2. State Pfluger's law.
3. Which kind of cleavage is found in *Amphioxus*?
4. Define 'Cells of Rauber'.
5. Which kind of cleavage is found in case of nematodes?
6. What does Chemodifferentiation involves?

5.4 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

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1. Cleavage was first observed by Swammerdam in 1738 in the egg of frog.
2. It states that the spindle elongates in the direction in which least resistance is offered.
3. Holoblastic cleavage is found in *Amphioxus*.
4. Trophoblast cells overlying the embryonal knob are called as Cells of Rauber.
5. Rotational cleavage is found in case of nematodes.
6. Chemodifferentiation involves physiological changes, it restricts changes in potency upon many localized cellular areas. As a result various future organs and parts of organs have their respective fates rigidly and irrevocably determined at the end of gastrulation.

5.5 SUMMARY

- Just after the fertilization, the most crucial event in the lifetime of an organism is the process of sequential mitotic divisions known as 'cleavage'.
- Process of cleavage was first observed by Swammerdam in 1738 in the egg of frog. First two cleavage planes in case of toad's egg were described by Spallanzani in 1780.
- The mechanical agent of this division is the mitotic spindle, with its microtubules composed of tubulin (the same type of protein that makes up the sperm flagellum).
- The mitotic spindle and contractile ring are perpendicular to each other and the spindle is internal to the contractile ring. The contractile ring creates a cleavage furrow, which eventually bisects the plane of mitosis, thereby creating two genetically equivalent blastomeres.
- The actin microfilaments are found in the cortex of the egg rather than in the central cytoplasm.
- Radial cleavage mostly observed in 8- celled stage where the upper blastomeres lie directly above the lower four cells.
- When the mitotic apparatus is inclined /tilted in clockwise direction then after third cleavage the upper micromeres is displaced obliquely to the right side of lower tier of micromere.
- Consequenlty complete embryo will be formed only if all the blastomeres remain adhered together; if any of the blastomeres looses the contact with another then their fate deciding ability also gets lost.

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- The fertilized egg in most cases contains yolk, which are inert bodies. During division these bodies exert mechanical influences.
- The incidence of cleavage provides unique opportunity to study the mechanism of cell division and specially the role of different cell organelles during division.
- The egg of frog is telolecithal with a considerable amount of yolk localized towards the vegetal pole.
- The cleavage is holoblastic in nature, but differs considerably from that of *Amphioxus* because of larger quantity of yolk.
- The first cleavage plane is meridional which occurs at about 3-3½ hours after fertilization.
- The four blastomeres thus produced are not qualitatively identical, because the grey crescent material is present in two of the four blastomeres.
- Each blastomere contains dark pigment at the animal pole and yellowish yolk towards the vegetal pole.
- The third cleavage is latitudinal and occurs at right angles to previous cleavage planes but passes slightly above the equator.
- In *Amphioxus*, the cleavages occur in a synchronous fashion, while in frog considerable degree of irregularities (asynchronism) appears in later stages.
- At the eight-celled stage, a small space makes its appearance between the four micromeres.
- The floor of the blastocoel is formed of macromeres.
- Typical meroblastic cleavage occurs in chick, where the segmentation activity is restricted only at the blastodisc or germinal disc.
- This furrow cuts across the blastodisc and passes towards the vegetal pole but does not reach the pole.
- Soon a cleft appears which separates the disc in the middle from the underlying yolk.
- The new cavity in between is known as sub-germinal space. This disc is then termed as blastoderm, the cells of which still continue to divide.
- Chemo-differentiation apparently occurs through inductive action.
- Chemical basis of differentiation or chemodifferentiation of any cell is based on the various differentiation or diversification products or enzymes which direct the synthesis of organic compounds that imparts the uniqueness to cell.

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5.6 KEY WORDS

- **Pfluger's law**: It is a law that states that the spindle elongates in the direction of least resistance.
- **Balfour's law**. It is a law that states that the rate of cleavage tends to be governed by the inverse ratio of the amount of yolk present, in holoblastic cleavage. The yolk tends to impede division of both the nucleus and the cytoplasm.
- **Sack's law**. According to this law cells tend to divide into equal parts and each new plane of division tends to bisect the previous plane at right angles.
- **Vertical plane of cleavage:** It is a state when a furrow passes in any direction (does not pass through the median axis) from the animal pole towards the opposite pole.
- **Equatorial plane of cleavage:** In this type of cleavage plane divides the egg halfway between the animal and vegetal poles and the line of division runs at right angle to the median axis.
- **Meroblastic cleavage**: In this type of cleavage, mitotic divisions occur only in the metabolically active cytoplasm which remains confined to the animal or the peripheral region of the egg.
- **Superficial cleavage:** This type of cleavage is the characteristic feature of centrolecithal eggs of insects.
- **Discoidal cleavage:** This type of cleavage is the characteristic feature of (meiolecithal eggs) reptiles, birds and other egg laying mammals.

5.7 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short Answer Questions

1. Write a note on the comparative account of various patterns of cleavage in different types of eggs.
2. What are actin microfilaments? Describe their existence.
3. Discuss the terms:
   - Pfluger's law
   - Balfour's law
   - Hertwig's law
   - Equatorial plane of cleavage
4. Differentiate the types of cleavage on the basis of yolk.

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5. What are the chemical changes during cleavage?
6. Write a note on the types of cleavage on the basis of potentiality of blastomeres.

Long Answer Questions

1. Write a detailed note on chemodifferentiation. From your learning of the text, explain the statement: 'Chemo-differentiation apparently occurs through inductive action.'
2. Discuss the importance of cleavage in embryonic development?
3. Write a detailed note on the different types of cleavage. Also list suitable examples for the same.
4. Elaborate the process of cleavage. Support your answer with your learning of the text.
5. Diagrammatically explain occurrence of cleavage in various oganisms studied by you.
6. 'Typical meroblastic cleavage occurs in chick, where the segmentation activity is restricted only at the blastodisc or germinal disc.' Explain.

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UNIT 6 BLASTULATION AND GASTRULATION IN FROG AND CHICK

Structure

6.0 INTRODUCTION

The blastulation is the formation of the blastula and blastocoels following the morula stage. Blastulation is the process following the morula and precedes the gastrulation. It entails cleavage resulting in a blastula consisting of about 128 cells. It is marked by the presence of a balstocoel. The blastula (Gr. blastos, sprout) is a hollow sphere of cells, referred to as blastomeres, surrounding an inner fluid-filled cavity called the blastocoel formed during an early stage of embryonic development in animals.

The blastocyst contains an embryoblast (or inner cell mass) that will eventually give rise to the definitive structures of the fetus, and the trophoblast, which goes on to form the extra-embryonic tissues. During the blastula stage of development, a significant amount of activity occurs within the early embryo to establish cell polarity, cell specification, cell differentiation, axis formation and regulate gene expression. The study of the blastula and of cell specification has many implications on the field of stem cell research as well as the continued improvement of fertility treatment. Blastocoel cavity performs major functions such as: it enables cell migration during the process of gastrulation, prevents or inhibits the interaction between the cells present beneath and above the blastocoels, and it also inhibits or prevents the contact of vegetal cells (macromeres) that are destined to become endodermal cells and ectodermal (skin and nerves).

NOTES

In this unit, you will study about blastulation and its types, highlight gastrulation in frog and chick, and the mechanism of morphogenetic movement.

6.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand blastulation and its types
- Know the mechanism of gastrulation and significance
- Discuss vegetal rotation and invagination of bottle cells
- Explain the formation of endoderm
- Learn about the morphogenetic movement

6.2 BLASTULATION

Embryo development begins with a sperm fertilizing of an egg to become a zygote which undergoes many cleavage to develop into a ball of cells called a morula. Only when the blastocoele is formed does the early embryo become a blastula. The blastula precedes the formation of the gastrula in which the germ layers of the embryo form. A common feature of a vertebrate blastula is that it consists of a layer of blastomeres, known as the blastoderm, which surrounds the blastocoele. In mammals the blastula is referred to as a balstocyst.

Embryonic stem cells are a field which, though controversial, has tremendous potential for treating disease. The blastomeres behave as pluripotent stem cells which can migrate down several pathways, depending on cell signaling. By manipulating the cell signals during the blastula stage of development, various tissues can be formed. This potential can be instrumental in regenerative medicine for disease and injury cases. In-Vitro Fertilization (IVF) involves implantation of a blastula into a mother's uterus. Blastula cell implantation could serve to eliminate infertility.

The improvement of microscopes, staining methods and microtomes helped those documents provide detailed descriptions of embryonic stages of chick development. With the help of researchers such as Rauber, Haeckel, Hamburguer and Hamilton, people now understand that chick gastrulation begins approximately seven to eight hours after fertilization. In the chick epiblast, a totipotent primordial cell layer, cells begin to rearrange at the posterior end. Those cells migrate inward to form the primitive streak, a midline thickening of the epiblast. During that time, the epiblast is separated from the hypoblast, a deeper layer of cells in the blastoderm, by the blastocoel, a fluid filled cavity. Future endoderm cells are the first cells to pass through the primitive streak. Those cells displace the hypoblast cells moving them towards the anterior pole of the embryo.

Next, Hensen's node, a mass of cells lying at the most anterior end of the primitive streak, drives elongation towards the posterior end of the embryo. The order in which the cells enter the blastocoel through Hensen's node determines which of the three germ layers they will become in the future embryo. As the embryo continues to grow and proliferate, Hensen's node regresses, leaving behind the notochord and signaling the beginning of neurulation, which is the formation of the central nervous system.

As the process of cleavage proceeds numerous number of blastomeres increases and all the blastomeres undergo a rearrangement. Intensity of adhesion between the blastomeres also get increased and all the blastomeres arrange themselves into a true epithelium. So formed epithelium could be single cell layered or multi cell layered and this epithelium is known as blastoderm. During this rearrangements of blastomeres a fluid filled cavity develops called as blastocoel in the centre of blastoderm. This hollow, spherical cellular stage of embryonic development is called blastula and the process of formation of blastula is called as blastulation (Refer Figure 6.1).

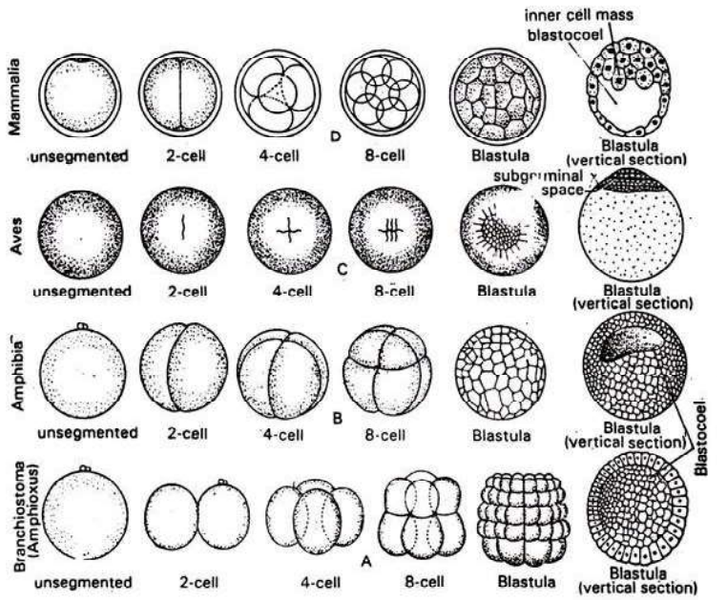

Fig. 6.1 *Showing Comparison of Blastulation in Different Chordates*

Different Types of Blastula

The blastula of various groups of animals differs in form and structure depending upon a variety of factors such as the size of the amount and distribution of yolk etc. (Refer Figure 6.2). Different group of animals are characterized by the presence of different types of blastula, as follows:

- **Coeloblastula**: It is a hollow blastula containing a large spacious blastocoel. Usually, the blastocoel is filled with a fluid containing mucopolysaccharides.

The blastula resulting from holoblastic equal cleavage, as in case of echinoderms and amphioxus, is called equal coeloblastula. In this case, the blastoderm is single layered. Holoblastic unequal cleavage, as found in frog, results in unequal coelobtastuta. It has a blastocoel displaced towards the animal pole and a multilayered blastoderm.

- **Stereoblastula**: This type of blastula is composed of an aggregate of larger sized and relatively lesser number of cells without or with extremely small blastocoelic space in the centre. Stereoblastula occurs in a variety of animals such as insects, some worms like Nereis, mollusks like Cripidula, gymnophionan amphibians and certain fishes.
- **Discoblastula**: Discoblastula consists of a disc shaped mass of blastomeres overlying a large yolk mass. This blastula is the result of meroblastic discoidal cleavage as in most fishes, reptiles and birds. There is no blastocoel, instead a slit like cavity called subgerminal cavity appears in between the blastoderm and the yolk mass
- **Blastocyst**: It is the blastula stage of mammals; consists of a hollow spherical vesicular blastula, containing an inner cell mass at the animal pole. The embryo proper develops from the inner cell mass. The outer single layer of cells which encloses the blastocoel is called the trophoblast. The trophoblast establishes relations with uterine wall and helps in nutrition of the developing embryo.
- **Periblastula**: A stage in the embryonic development of most arthropods having centrolecithal eggs. The periblastula is a vesicle whose wall consists of one layer of cells and whose cavity is filled with unbroken yolk. It is formed as a result of the superficial segmentation of the egg.

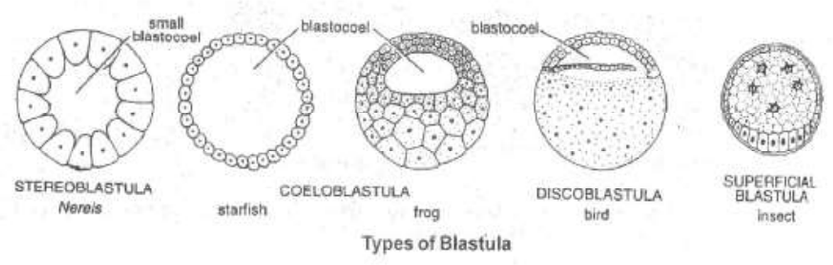

Fig. 6.2 *Schematic Presentation of Different Blastula*

Functions of Blastocoels

Blastocoel cavity performs major functions such as:

- It enables cell migration during the process of gastrulation
- It prevents or inhibits the interaction between the cells present beneath and above the blastocoels
- It also inhibits or prevents the contact of vegetal cells (macromeres) that are destined to become endodermal cells and ectodermal (skin and nerves)

As the cleavage process is regular and synchronus which means all the blastomers divide simultaneously however in case of amphibians there is abrupt change in duration and synchrony of the cell cycle that is known as Mid Blastula Transition (MBT) whereas in other animals the transition from a cleavage to a somatic cell cycle is gradual.

Mid Blastula Transition (MBT)

In case of *Xenopus laevis* major transition occurs that involves the initiation of transcription, elongation of cell cycle, increase in cell movement and activation of genes as the nuclear genes are not activated until late 12^{th} division cell cycle. At this time, embryo experiences a mid-blastula transition (strengthening before gastrultion). Different genes begin to be transcribed in different cells, cell cycle acquire gap phase and blastomere acquires the capacity to become motile. It is thought that some factors in the egg are absorbed by newly made chromatin and time of mid blastula transition can be changed experimentally by altering the ratio of chromatin to cytoplasm in the cell.

There are two events that trigger mid blastula transition, which are as follows:

- **First** event involves chromatin modification: certain promoters are demethylated, allowing transcription of genes during late blastula stages. The loss of methylation on the gene promoters that are activated at MBT. This demethylation is not seen on those promoters that are not activated during MBT.
- **Second** is methylation of Lysine 4 on Histone 3; also seen on 5' end of many genes during MBT. Modification of certain promoter and their asociated nucleosome plays vital role in regulating the timing of gene expressions at the MBT.

Various transcription factors such VegT protein formed in the maternal cytoplasm/ vegetal cytoplasm bind to promoters and initiate the new transcription. This is how amphibian blastula prepares itself the various gastrulation movements for the formation of three germ layers.

6.3 GASTRULATION IN FROG

Gastrulation is an early stage in embryo development in which the blastula reorganizes into the three germ layer: the ectoderm, the mesoderm, and the endoderm. Gastrulation occurs after cleavage but before neurulation and rganogenesis. E. Haeckel coined the term; 'gaster', meaning stomach in Latin, is the root for gastrulation', as the gut is one of the most unique creations of the gastrula. Gastrulation can be defined as the dynamic process during which the major, presumptive organ-forming areas of the blastula become rearranged and reorganized in a way which permits their ready conversion into the body plan of the particular species (Nelsen, 1953).

An amphibian embryo containing 16 to 64 cells is commonly called as morula. At 128th celled stage the blastocoel cavity become apparent; then the embryo is known as Blastula. During the process of cleavage the cell adhesion molecules (EP-Cadherin) are present which keep the cleaving blastomeres together. However there will be no formation of EP-Cadherin molecule if no mRNA is supplied to the oocyte cytoplasm as the message gets destroyed by antisense oligonucleotides and hence the adhesion between blastomeres get reduced ultimately leads to the obliteration of the blastocoel. Generally the blastomeres present at the animal hemisphere gives rise to ectoderm, the blastomeres present at the vegetal hemisphere gives rise to the endoderm and the cells present beneath the blastocoel cavity give rise to mesoderm. Cells that are present at position opposite to the point of sperm entry will become neural ectoderm, notochord mesoderm and the pharyngeal endoderm.

In amphibians process of gastrulation starts with the movement of cells in Grey Crescent area (area opposite to entry of sperm in egg and present just below the equator) (Refer Figure 6.3). From this region the cells called as Bottle cells invaginate to form slit like aperture called as Blastopore. These bottle cells change their shape dramatically. Main body of each cell displaced towards inside of embryo while maintaining the contact with outside surface through slender neck. Gastrulation begins in the Marginal zone (region surrounding the equator where the animal and vegetal hemisphers meet).

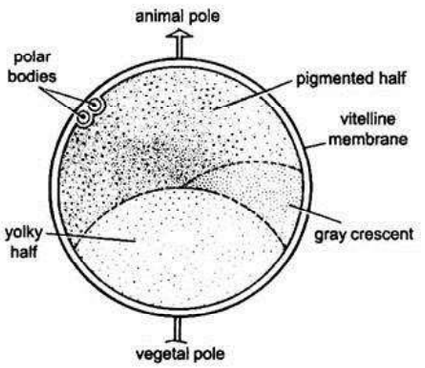

Fig. 6.3 *A Fertilized Egg of Frog Showing Area of Gray Crescent*

Vegetal Rotation and Invagination of Bottle Cells

At least two hours before the bottle cells are formed, internal cells rearrangement occurs which propel the cells from dorsal floor of blastocoel towards animal cap. This vegetal rotation places the prospective pharyngeal endoderm cells adjacent to blastocoel and immediately above the involuting mesoderm. Due to this movement of bottle cells, a slit like aperture is formed and called as Blastopore or Blastopore lip. Animal cells undergo epiboly, produces stem of cells that converge at and become dorsal blastopore lip (Refer Figure 6.4).

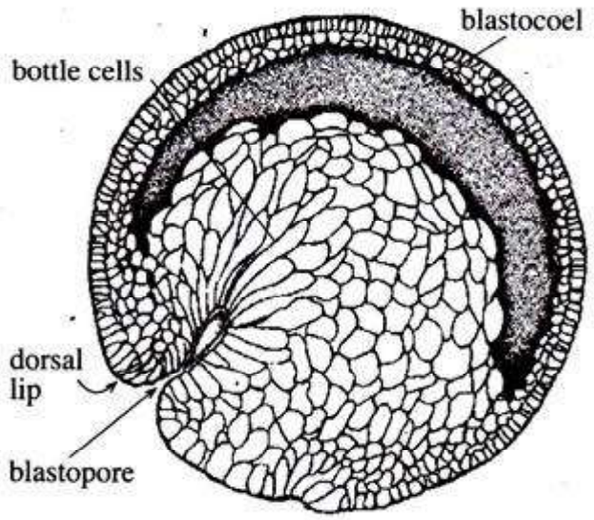

Fig. 6.4 *Showing the Flask-Shaped Bottle Cells Moving into the Interior at the Blastopre in an Advanced Amphibian Gastrula*

Involution at the Blastopore Lip

Here the superficial layer of the marginal cells gets pulled inward to form endodermal lining of archenteron (primitive gut). If there is removal of deep Involuting Marginal Zone (IMZ) cells, it stops archenteron formation. First cell that compose dorsal blastopore lip and enter the embryo are the prospective pharyngeal endoderm. As these cells passes into interior of the embryo, dorsal blastopore lip becomes composed of cells that involute into embryo Pre-chordal plate. Cells of pre-chordal plate transcribe *goosecoid* gene, its transcription activates numerous genes that control head formation. Next, the cells involuting through dorsal blastopore lip are the chordamesoderm cells. Chordamesoderm cells will form the notochord which is the transient mesodermal rod that plays a crucial role in inducing and pattern formation of nervous system. In the formation of spinal cord X Bra gene expressed by chordamesodermal cells plays an important role. As the new cell enters the embryo, blastocoel is displaced to side opposite the dorsal lip and this dorasal blastopore lip expands laterally and venterally to form venteral mesoderm (Refer Figure 6.5).

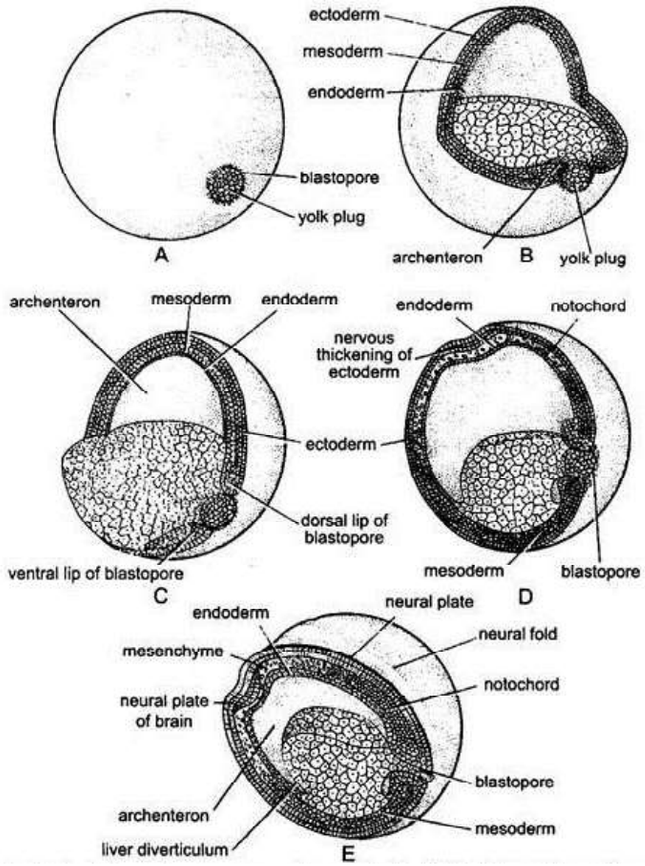

Fig. 6.5 *Gastrulation in Frog: Gastrula Surface View (A), Early Gastrula with Yolk Plug (B, C), Late Gastrula with Germ Layers (D, E)*

Convergent Extension of Dorsal Mesoderm

Widening of Blastopore lip finally develops into venteral blastopore lip. With the formation of venetral lip, Blastopore has formed ring around large endodermal cells that remains exposed on vegetal surface. Remaining patch of endoderm is called as **Yolk Plug**. Involuting marginal zone cells are several layer thick, these several layer of deep IMZ cells intercalate readily to form one thin broad layer. This radial intercalation extends involuting marzinal zone cells vegetally. Outer most superficial cells called Non- involuting marzinal zone cells involute into embryo and initaiate second type of intercalation when the deep cells reach the blastopore lip. Second type of intercalation causes convergent extension along medio-lateral axis that integrate mesoderm stream to form long narrow bands. This mesodermal stream continues to migrate towards animal pole. These radial and medio-lateral intercalations of the deep layers of the cell are responsible for the continued movement of mesoderm into embryo.

The **forces to driven convergent extension are:** Polarized cell cohesion, Adhesion proteins (paraaxial protocadherin, axial protocadherin), and Calcium

flux. As the mesodermal movement progresses, convergent extension continue to narrow amid lengthening the Involuting Marzinal Zone (IMZ) and this movement of cells is autonomous in nature. Region where remant of blastopore and the endoderm meets ectoderm, become Anus.

Epiboly of Prospective Ectoderm

During gastrulation, the animal cap and non-involuting marginal cells expand by epiboly to cover the entire embryo. These cells will form surface ectoderm. The important mechanism of epiboly in frog is:

- Increase in the cell number through numerous divisions coupled with the integration of several deep layers of cells into one single layer
- Involves assembly of fibronectin into fibrils by the blastocoel roof and this fibrillar fibronectin is critical in allowing the vegetal migration of animal cap cells and allows the enclosure of embryo (Refer Figure 6.6).

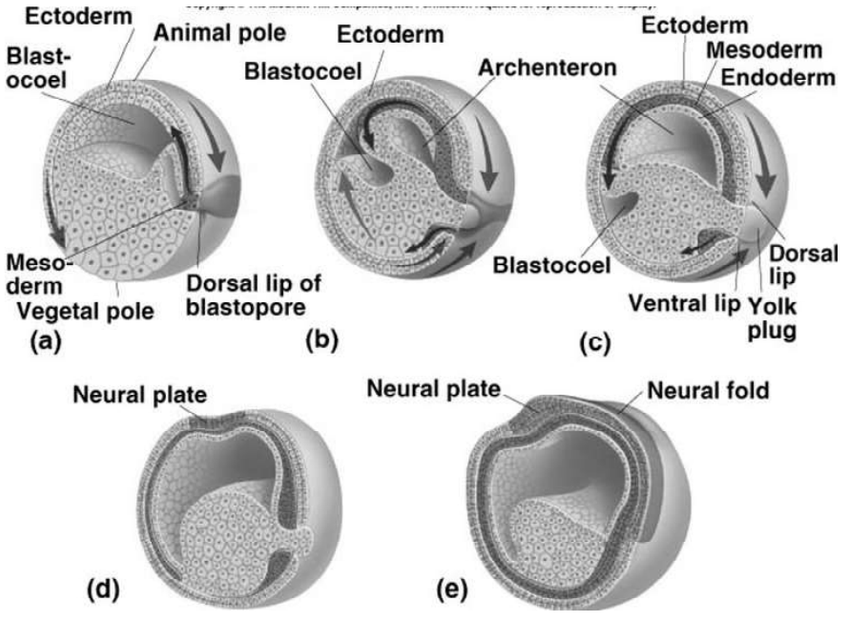

Fig. 6.6 *Schematic diagram of Gastrulation and Morphogenetic Movement in Frog*

Check Your Progress

1. What does embryo development begin with?
2. Where does stereoblastula occur?
3. What does a discoblastula consists of?
4. What happens during gastrulation?

NOTES

6.4 GASTRULATION IN CHICK

Gallus gallus (domestic fowl) is a major model system in embryology. It was one of the first organisms used for developmental research in the nineteenth century because the egg could be opened and the development of embryo inside could be seen without the use of a powerful microscope. The embryo's large size and the ability to survive under surgical manipulation gave the chick an advantage over other model systems. There were two suggested explanations of chick gastrulation. The first suggested that the mesoderm formed from the epiblast; the early stage totipotent layer of cells and the mesoderm then differentiated into the endoderm. The other suggestion was that the epiblast and endoderm developed together first, followed by the mesoderm. It wasn't until August Rauber discovered that the two-layered chick embryo is a blastoderm, a flat layer of embryonic cells that folds several times to become the later stages of an embryo; that gastrulation began to be understood. Rauber emphasized that the mesoderm initiates the ectoderm and endoderm to differentiate and that the blastoderm was essentially the canvas for gastrulation.

In chick embryos, the ectoderm, mesoderm, and endoderm cells ultimately give rise to different tissues and organs. Ectoderm cells generate the skin and neural tissue. Endoderm cells become the lining of the gastrointestinal and the respiratory tracts. Mesoderm cells differentiate into the circulatory system, kidneys and skeletal compartments among many other features. Those tissues and organs are created during organogenesis. Gastrulation of the chick is a crucial step in development that turns a simple multi-cellular embryo into a complex fully functional organism. Regardless of many researchers involved, questions still remain regarding the mechanisms of induction and genetics involved in the cells movements that occur during gastrulation. The chick embryo is still used today by researchers who hope to answer those developmental questions.

Mechanism of Gastrulation in Chick

Cleavage is meroblastic or discoidal and is confined only to be germinal disc or blastodisc. It does not segment the yolk of the polylecithal egg and it is later eventually surrounded by the growing tissues of the embryo. First two cleavages are at right angles to each other in the centre of blastodisc. These cleavage furrows do not cut the germinal disc completely through in the vertical plane. The third set of cleavage furrows is vertical, cutting across the second set of vertical furrows. The fourth cleavage furrow is also vertical and circular cutting across all the cleavage furrows, forming eight central blastomeres which are surrounded by eight marginal blastomeres. Thus, these cleavage furrows separate the daughter central blastomeres from each other, but not from the yolk. The central blastomeres are continuous with the underlying yolk at their lower ends. The marginal blastomeres are continuous with the uncleaved cytoplasm at their outer edges. Further cleavages are irregular. The central cells divide more rapidly. The marginal cells also divide by the

appearance of new horizontal and radial furrows. The newly formed inner cells of marginal blastomeres are added to the central cells, resulting in the increase of volume of this area. The radial furrows extend peripherally and these peripheral cells are still continuous with the uncleaved peripheral cytoplasm, (Refer Figure 6.7).

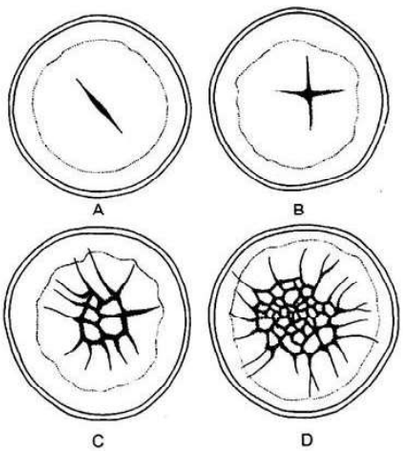

Fig. 6.7 *Surface view of germinal disc and cleavage in chick's egg.*

In later stage of cleavage, the blastomeres of the central area become separated from the underlying yolk due to the appearance of a horizontal cleavage in these cells. This cleavage extends peripherally cutting the inner ends of the blastomeres. Thus, a space also appears in the beginning; beneath the central cells which also extends peripherally as the horizontal cleavage extends outward. The cavity beneath the central cells, i.e., in between central cells and yolk is called the subgerminal cavity, which is filled with a fluid diffused from the albumen through vitelline membrane. Thus, due to further cleavage the blastodisc becomes cellular, called the blastoderm- a round disc, 5 to 6 cells deep in the centre but only 1 to 2 cells deep at the periphery. The appearance of subgerminal cavity separates the blastoderm from the underlying yolk, but the marginal cells remain overlapping the yolk. The embryo is now called the blastula stage (Refer Figure 6.8).

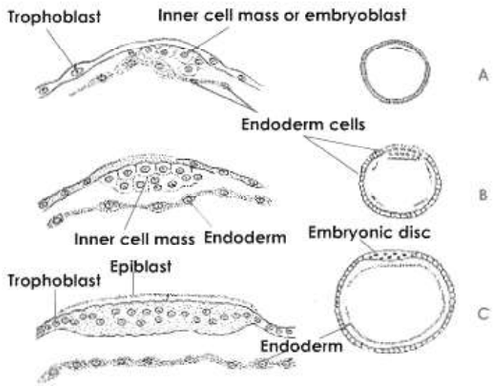

Fig. 6.8 *Formation of Germ Layers in Embryo*

NOTES

At blastula stage the embryo reaches in the uterus. It may be compared to the blastula of *Amphioxus* and frog, its sub-geminal cavity is equivalent to the blastocoel, the blastoderm is the animal pole, and the yolk is the vegetal pole. During later part of cleavage, about 12 to 14 hours after the egg reaches the uterus or 6 to 8 hours before the egg is laid, some cells on the inner or under side of the blastoderm become detached or delaminated from the blastoderm and fall on the floor of subgerminal cavity due to presence of relatively more yolk. The delamination of these yolky cells from the blastoderm starts at the posterior edge and spreads forward until whole blastoderm becomes free from yolky cells. As a result, the epithelial layer in the central region of blastoderm becomes thinner (few layers of cells) and transparent. Thus, this region is called the area pellucida because it seems to be transparent when viewed from the upper side. The peripheral part of blastoderm, the yolky cells is not delaminated (shed), so this part of the blastoderm seems to be opaque, because beneath these cells blastocoel is not present. This region of blastoderm is, thus, called area opaca. These delaminated cells at the posterior edge of area pellucida gradually link up with each other, forming a, continuous layer of flattened cells, which extends anteriorly. This layer is called the hypoblast and the upper layer is the epiblast containing ectoderm and mesoderm cells. Hypoblast is exclusively composed of endoderm cells. The egg is laid by the female about the time the blastula is formed or even a little later, (Refer Figure 6.9).

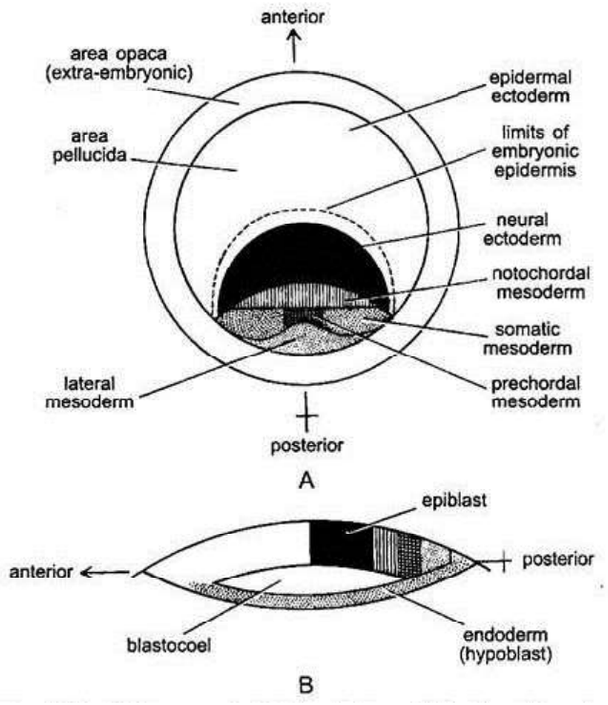

Fig. 6.9 *Fate Maps of Chick Blastoderm: Surface View (A); Section of Discoblastula (B)*

Presumptive Fate Maps of Blastula

Fate maps of the blastula of chick have been prepared by using the vital stains such as carmine or carbon (charcoal) particles or radioactive thymidine. It shows that blastomeres of area opaca do not form any part of the embryo proper, they form only extra-embryonic membranes. The epiblast and hypoblast of area pellucida have different fates in the course of embryonic development. The fate maps prepared by the use of tritiated thymidine have shown the following structures in epiblast. In the centre of area pellucida lies a small area destined to produce the notochord. Posterior to it, in the median plane is found an elongated oval area of presumptive endoderm which will form the gut (Refer Figure 6.10).

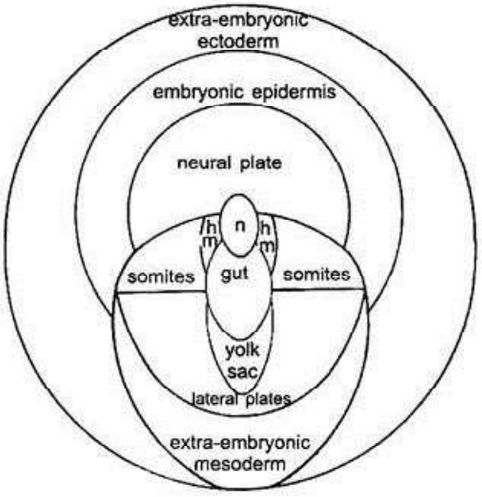

Fig. 6.10 *Schematic Representation of Fate Map of Epiblast of a Chick*

Further toward the posterior edge of area pellucida lies the extra-embryonic endoderm which forms the lining of yolk sac. To the right and left of presumptive notochord and endoderm, posterior to extra-embryonic endoderm lie various subdivisions of presumptive mesoderm, i.e., prechordal plate or head mesoderm, mesodermal somites, lateral plate mesoderm and extra-embryonic mesoderm. The anterior half of epiblast is the presumptive ectoderm containing central presumptive neural plate area, anterior to it is the presumptive embryonic epidermis and outer to it is the extra-embryonic ectoderm in the form of a complete ring.

6.5 MORPHOGENETIC MOVEMENTS

During gastrulation, cells from one region of embryo move to another to take up their future fateful position. Two terms, emboly and epiboly which are quite opposite in their meanings, are generally applied to explain the process of movement. Emboly means the throwing in or insertion of cells and epiboly signifies its extending. The movement of cells establishes a particular form and is involved in organ formation

in embryo, so this movement is designated as the morphogenetic movement. Formation of primitive streak and head process is due to emboly.

Formation of Primitive Streak

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Various prospective mesodermal and endodermal cells forming notochord of the epiblast converge toward the posterior edge of the area pellucida and form a conical thickening in the midline, called the initial primitive streak. It appears after 6 to 7 hours of incubation. The primitive streak grows anteriorly because of proliferation of its own cells as well as of the addition of cells that migrate to it from anterior and lateral parts of area pellucida. The elongated axis of the primitive streak marks the antero-posterior axis of the future embryo. It, thus, eventually extends to, about three fifths of the entire length of area pellucida. This is the fully developed definitive primitive streak and it is usually completed after 18 to 19 hours of incubation. The area pellucida also becomes pear-shaped. Along the middle of the primitive streak, when it is fully developed runs a narrow furrow, the primitive groove. At the anterior end of the primitive streak there is a thickening, the primitive knot or Hensen's node. The centre of Hensen's node is excavated to form a funnel-shaped depression. The movements in the blastoderm leading to the final placement of cells in the hypoblast and to the formation of the primitive streak in the epiblast may be called pregastrular movements (Refer Figure 6.11).

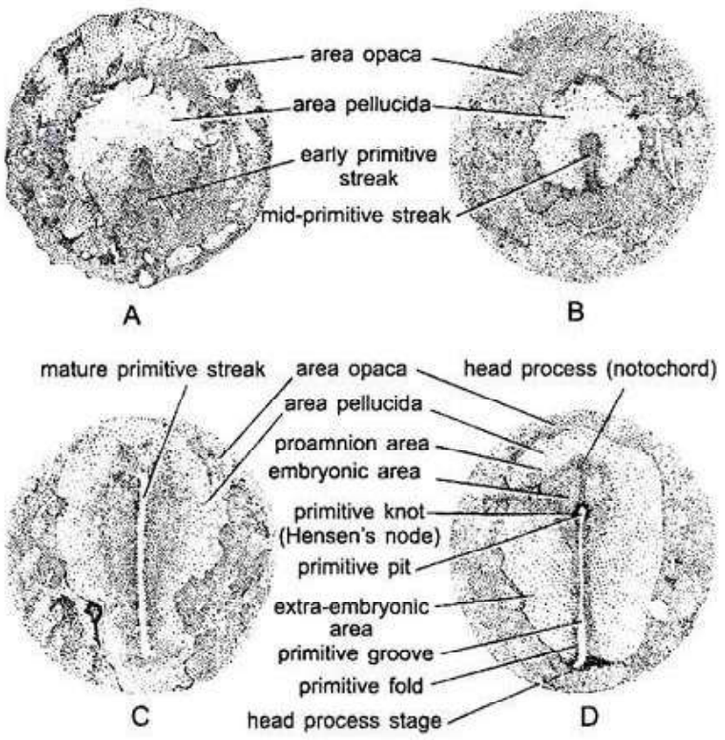

Fig. 6.11 *Surface View of Chick Blastodem showing Development of Primitive Streak during Gastrulation*

Invagination and Involution

At the stage of short primitive streak, the cells of the blastoderm already begin to migrate (invaginate and involute) into the blastocoel cavity between epiblast and hypoblast. Immigrating cells are replaced by more epiblast cells converging toward the streak area. The inward migrating cells also spread out sideways and forward from the anterior end of primitive streak. The notochordal cells immigrate through primitive pit. Endodermal cells invaginate through that part of the streak which lies just behind primitive pit. The mesodermal cells of somites just follow the path of endodermal cells. Whereas the lateral plate mesoderm cells invaginate through the middle section of primitive streak, but only after the disappearance of endoderm from the area pellucida. The extra-exbryonic mesoderm (of the yolk sac) immigrates through the posterior part of primitive streak. Meanwhile, some hypoblast cells expand into the area opaca to become extra-embryonic endoderm (the lining of yolk sac), while other hypoblast cells attach to mesodermal and notochordal cells are carried along by the latter's migration.

Formation and Development of Head Process

Prospective notochoral cells converge on the node, sinks through it and then passes directly forward as a tongue of tissue known as head process or notochord process. The midline area of notochordal tissue develops into a rigid rod, anterior to the receding primitive streak. As the streak regresses posteriorly, the embryo develops anterior to it. The head process consists of a thick central mass of cells and more diffused lateral wings. In the beginning it is also blended in the midline with the hypoblast. The thicker central portion forms the definitive notochord, whereas the lateral wings form the paraxial (somitic) mesoderm. With its differentiation, the notochord becomes detached from the hypoblast below, except at the extreme end. Thus, the head process stage is completed at about 20 to 25 hours of incubation. Gastrulation is also completed at this stage.

Disappearance of Primitive Streak

With the gradual disappearance of endodermal, notochordal and mesodermal cells from the primitive streak, it begins to shrink from anterior towards posterior side and its remains are partly included in the tail bud and partly into the cloacal region of the embryo.

Formation of Endoderm

The first cells that migrate through the anterior part of streak form the endoderm. As the Hensen's node recedes backward and the notochordal process elongates, the presumptive endoderm of the middle and posterior part of the gut, located just behind the node, migrate inside as an endodermal strip beneath the notochord. The original hypoblast at the floor of the blastocoel contribute a very less amount to the gut, the upper migrated endodermal cells form the major part of the gut. In chick no archenteron is formed during gastrulation.

Fully Formed Gastrula

Gastrula is fully formed when primitive streak completely disappears. The fully formed gastrula consists of three germ layers-ectoderm, chorda-mesoderm and endoderm. The ectoderm and chorda-mesoderm remain in continuity along the axis of primitive streak. The endoderm is also united with the mesoderm and ectoderm at the anterior and posterior end of streak, (Refer Figure 6.12).

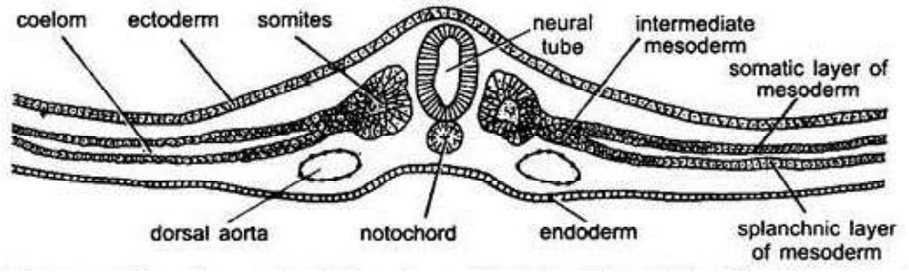

Fig. 6.12 *A Cross Section of an Early Chick Embryo*

Check Your Progress

5. What was the first organisms used for developmental research in the nineteenth century?
6. What is cleavage?
7. What is the formation of primitive streak due to?
8. What are fate maps of the blastula of chick prepared by?

6.6 FORMATION OF NEURAL TUBE (NEUROGENESIS) IN CHICK

The ectoderm, anterior and lateral to the head process, becomes thickened to form the neural plate while the gastrulation process is going on. The neural plate appears in the brain region. As Hensen's node recedes farther and farther, parts of the neural plate become differentiated and the anterior parts of the neural plate proceed to close into a tube, the neural tube. The formation of neural tube occurs due to sinking in of the neural plate due to which a neural groove is formed along its longitudinal axis. Its elevated margins are called neural folds, which rise up and grow toward the midline and fuse to form the neural tube, (Refer Figure 6.13).

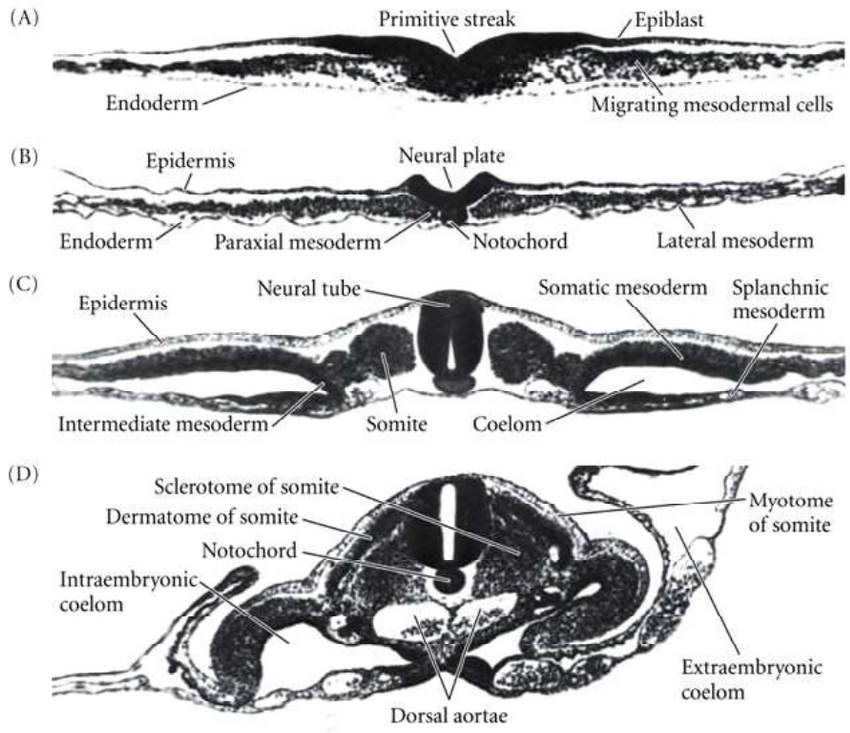

Fig. 6.13 *Development of Neural Tube, Notochord and Mesodermal Components in Chick Embryo*

Formation of Notochord and Mesoderm

While the neural plate is folding into the neural tube, the chorda-mesoderm is also differentiating. Its most anterior part, the prechordal plate mesoderm gives rise to the mesenchyme of head and behind it the notochordal cells become separated from the rest of the adjoining sheets of mesoderm and get differentiated into notchord (Refer Figure 6.14). On either side of notochord are three longitudinal strands or sheets of mesoderm:

- Epimere, axial or somatic mesoderms are the thicker dorsal medial strands, which merge anteriorly into the mesenchyme of head.
- Mesomeres or intermediate mesodermal strands are located lateral to the axial mesoderm. They are thin in an early stage.
- Hypomeres or lateral plate mesoderm lies beneath the mesomere.

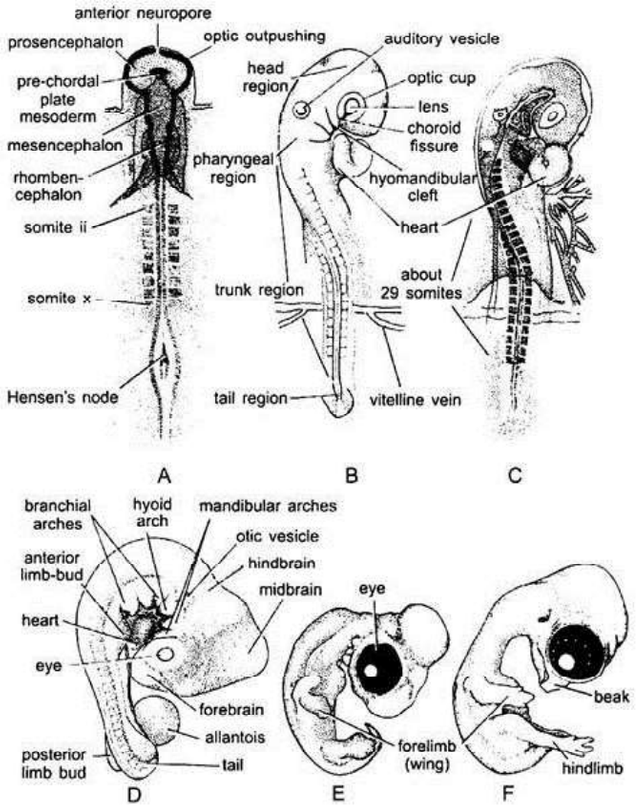

Fig. 6.14 *Post Neural Development of Chick Embryo*

The above Figure 6.14 shows the post neural development of chick embryo: stained stage 10 (A); surface view stage 16 (B); anatomy of stage 16 (C); stage 20 (D); stage 27 (E); stage 23 (F).

Check Your Progress

9. What path do the mesodermal cells of somites follow?
10. Where does the extra-exbryonic mesoderm immigrates through?
11. When is gastrula fully formed?
12. What is the reason for the formation of neural tube?

6.7 FLEXURE AND TORSION

When the head is formed it gets bent down ventrally with respect to the main axis of the embryo, this bending is called cranial flexure. Then the head at first and gradually the entire body turn sideways so that the embryo comes to lie on its left side over the yolk, this dextral twist is knows as torsion. The anterior end in its

cervical region becomes still more bent over towards its ventral surface. Various organs are formed on the third and fourth days of incubation, such as the nervous system, sense organs, four pairs of pharyngeal pouches out of which only the first three pairs meet the ectoderm to open as gill-clefts, five pairs of visceral arches, paired segmental somites heart and blood vessels.

Mechanism of Morphogenetic Movements

Morphogenetic movement is caused by large-scale and dynamic movement of embryonic cells. It rearranges the distribution of embryonic cells, thereby allowing the interaction between germ layers that previously existed separately. The first morphogenetic movement triggered is gastrulation, which forms the future digestive tract. Gastrulation is one of the most important morphogenetic movements in the formation of the basic tubular structure of animals. Morphogenetic movement consists of several basic cellular deformations and movements, including the invagination movement, in which contraction of one side of the embryo causes bending and encourages epithelial cells to extend inward into the embryo, the extension movement caused by the rearrangement of epithelial cells and the ingression movement of epithelial cells migrating into the embryo.

There are two types of morphogenetic movement:

- Emboly
- Epiboly

Emboly

The word emboly is derived from a Greek word meaning to throw in or thrust in. Emboly refers to the inward movement of the presumptive chorda-mesodermal and endodermal and their extension along the anteroposterior axis of the future embryo. This inward movement of cells is due to innate forces within various cell groups. It involves only the epiblast which contains cells of ectoderm, mesoderm and notochordal cells. It includes convergence, invagination, involution, Concrescence, ingression, delamination, extension, (Refer Figure 6.15):

- **Convergence and Divergence:** The migration of cells from the outside surface of the blastula to the external margin of the blastoporal lip is called convergence. Divergence is the opposite of convergence, where the cells diverge after getting aggregated. For example, the cells having involuted over the blastoporal lips into the interior, migrate away from that position to their future positions within the forming gastrula.
- **Invagination:** It implies an in-folding or in-sinking of a sheet of cells into the embryo. It results in the formation of a cavity surrounded by these in-folded cells. This process is executed in two ways: (a). Mechanical or passive in-folding of cells as seen in the lateral and ventral lip areas of the blastopore. (b). Active inward streaming or in-pushing of cells as exhibited by the dorsal lip region of the blastopore, into the blastocoelic space.

- **Involution:** The word involution means a "turning in" or inward rotation of cells. Involution is dependent much upon the migration of cells toward the blastoporal lip. These cells instead of piling up along the outer edges of the blastoporal lip or along the primitive streak, tend to move over the lip to the inside edge of the lip and are thus deposited on the inside of the embryo along the inner margin of the blastopore.
- **Concrescence:** Concrescence, the term used in older descriptions of gastrulation, denotes the movement of masses of cells toward each other, particularly in the region of the blastopore. It is used to imply the idea of fusion of cell groups. It probably does not occur.
- **Ingression:** When a cell or small groups of cells separate itself from other layers and migrate into the segmentation cavity within the developing body; then it is termed as ingression or poly-invagination. This is seen in the case of reptiles, birds and mammals where the mesodermal cells detach themselves from the primitive streak and migrate into the space between the epiblast and hypoblast.
- **Delamination:** Delamination is the splitting or separation of one sheet of cells either into two sheets or separation from other cell groups. The separation of notochordal, mesodermal and endodermal tissues from each other, during gastrulation of frog, to form discrete cellular masses, is an example of delamination. Another example is the formation of hypoblast in mammalians and birds.
- **Extension:** The extension of cellular masses also takes place in gastrulation. For example, the extension of the presumptive neural and epidermal areas externally and of the notochordal, mesodermal and endodermal cells after they have moved inward beneath the neural plate and epidermal material.
- **Infiltration:** During this process, cells of the blastoderm infiltrate near the bottom of the blastocoel to form a second layer as seen in the gastrulation of chick.
- **Cell Proliferation:** Cell proliferation implies an increase in the number of cells. It is intimately associated with the gastrulative process in Amphioxus, while in frog it is of lesser importance.

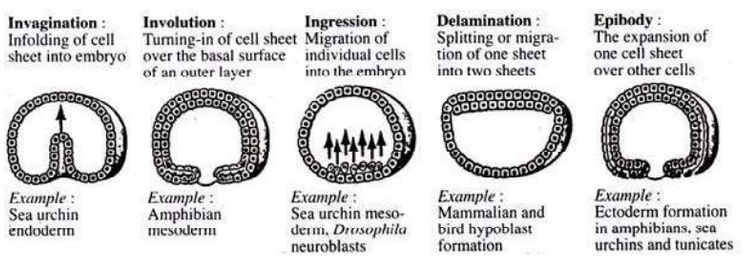

Fig. 6.15 *Types of Morphogenetic Movements during Gastrulation*

Epiboly

Epiboly is a morphogenetic process that is employed in the surface ectoderm of anamniotes during gastrulation to cover the entire embryo. It includes the overgrowth of ectoderm or epiblast and also of hypoblast. ***Epiboly*** *is defined as the movement of cells on the surface of the embryo. Epiboly is mainly classified under Morphogenetic movements. The movement of cells from one place to another to attain a new shape to the developing embryo is known as Morphogenetic movements.* This word Epiboly is derived from the Greek, meaning '*throwing on'* or '*extending upon'*. The ectodermal cells are involved in the epiboly. The ectodermal cells here mentioned: epidermal ectoderm and neuroectoderm. During Gastrulation, the ectodermal cells spreads out antero-posteriorly and as well as laterally. Hence, the entire gastrula staged embryo is gradually covered by ectodermal cells. While the presumptive mesodermal and endodermal cells are moving inward, the ectodermal precursors proliferate. Moreover, the ectodermal cells migrate to surround the yolk by epiboly. The enclosure of the yolk by the ectoderm (again reminiscent of the epiboly of amphibian ectoderm) is a Herculean task that takes the greater part of 4 days to complete. It involves the continuous production of new cellular material and the migration of the presumptive ectodermal cells along the underside of the vitelline envelope. Interestingly, only the cells of the outer edge of the area opaca attach firmly to the vitelline envelope. These cells are inherently different from the other blastoderm cells, as they can extend enormous cytoplasmic processes onto the vitelline envelope.

Check Your Progress

13. What is epiboly?
14. Where is the word 'Epiboly' derived from?
15. What is convergence?
16. What is the morphogenetic movement caused by?

6.8 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Embryo development begins with the sperm fertilizing of an egg.
2. Stereoblastula occurs in a variety of animals such as insects, some worms like Nereis, mollusks like Cripidula, gymnophionan amphibians and certain fishes.
3. Discoblastula consists of a disc - shaped mass of blastomeres overlying a large yolk mass.

4. During gastrulation, the animal cap and non-involuting marginal cells expand by epiboly to cover the entire embryo.
5. *Gallus gallus* was one of the first organisms used for developmental research in the nineteenth century.
6. Cleavage is meroblastic or discoidal and is confined only to be germinal disc or blastodisc.
7. Formation of primitive streak and head process is primarily due to emboly.
8. Fate maps of the blastula of chick have been prepared by using the vital stains such as carmine or carbon (charcoal) particles or radioactive thymidine.
9. The mesodermal cells of somites just follow the path of endodermal cells.
10. The extra-exbryonic mesoderm (of the yolk sac) immigrates through the posterior part of primitive streak.
11. Gastrula is fully formed when primitive streak completely disappears.
12. The formation of neural tube occurs due to sinking in of the neural plate due to which a neural groove is formed along its longitudinal axis.
13. Epiboly is a morphogenetic process that is employed in the surface ectoderm of anamniotes during gastrulation to cover the entire embryo.
14. The word Epiboly is derived from the Greek, meaning throwing on or extending upon.
15. Convergence is the migration of cells from the outside surface of the blastula to the external margin of the blastoporal lip.
16. Morphogenetic movement is caused by large-scale and dynamic movement of embryonic cells.

6.9 SUMMARY

- The blastulation is the formation of the blastula and blastocoels following the morula stage. Blastulation is the process following the morula and precedes the gastrulation. It entails cleavage resulting in a blastula consisting of about 128 cells. It is marked by the presence of a balstocoel.
- Embryo development begins with a sperm fertilizing an egg to become a zygote which undergoes many cleavage to develop into a ball of cells called a morula. Only when the blastocoele is formed does the early embryo become a blastula.
- Embryonic stem cells are a field which, though controversial, has tremendous potential for treating disease.
- Future endoderm cells are the first cells to pass through the primitive streak.
- Gastrulation is an early stage in embryo development in which the blastula reorganizes into the three germ layer: the ectoderm, the mesoderm, and the endoderm.

- Cells that are present at position opposite to the point of sperm entry will become neural ectoderm, notochord mesoderm and the pharyngeal endoderm.
- Chordamesoderm cells form the notochord which is the transient mesodermal rod that plays a crucial role in inducing and pattern formation of nervous system. In the formation of spinal cord XBra gene expressed by chordamesodermal cells plays an important role.
- As the mesodermal movement progresses, convergent extension continue to narrow amid lengthening the Involuting Marzinal Zone (IMZ) and this movement of cells is autonomous in nature.
- Ectoderm cells generate the skin and neural tissue. Endoderm cells become the lining of the gastrointestinal and the respiratory tracts.
- Gastrulation of the chick is a crucial step in development that turns a simple multi-cellular embryo into a complex fully functional organism.
- The chick embryo is still used today by researchers who hope to answer those developmental questions.
- Cleavage is meroblastic or discoidal and is confined only to be germinal disc or blastodisc.
- First two cleavages are at right angles to each other in the centre of blastodisc. These cleavage furrows do not cut the germinal disc completely through in the vertical plane.
- The epiblast and hypoblast of area pellucida have different fates in the course of embryonic development.
- Further toward the posterior edge of area pellucida lies the extra-embryonic endoderm which forms the lining of yolk sac.
- During gastrulation, cells from one region of embryo move to another to take up their future fateful position.
- The ectoderm and chorda-mesoderm remain in continuity along the axis of primitive streak.
- The ectoderm, anterior and lateral to the head process, becomes thickened to form the neural plate while the gastrulation process is going on.
- Morphogenetic movement is caused by large-scale and dynamic movement of embryonic cells.
- The word emboly is derived from a Greek word meaning to throw in or thrust in.
- The migration of cells from the outside surface of the blastula to the external margin of the blastoporal lip is called convergence.
- Divergence is the opposite of convergence, where the cells diverge after getting aggregated.

- The enclosure of the yolk by the ectoderm (again reminiscent of the epiboly of amphibian ectoderm) is a Herculean task that takes the greater part of 4 days to complete.
- In mammals the blastula is referred to as a balstocyst. The blastocyst contains an embryoblast (or inner cell mass) that will eventually give rise to the definitive structures of the fetus, and the trophoblast, which goes on to form the extra-embryonic tissues.
- The blastomeres behave as pluripotent stem cells which can migrate down several pathways, depending on cell signaling. By manipulating the cell signals during the blastula stage of development, various tissues can be formed. This potential can be instrumental in regenerative medicine for disease and injury cases.
- Discoblastula consists of a disc-shaped mass of blastomeres overlying a large yolk mass. This blastula is the result of meroblastic discoidal cleavage as in most fishes, reptiles and birds.
- Gastrulation is an early stage in embryo development in which the blastula reorganizes into the three germ layers: the ectoderm, the mesoderm, and the endoderm.
- Cleavage is meroblastic or discoidal in chick and is confined only to be germinal disc or blastodisc. It does not segment the yolk of the polylecithal egg and it is later eventually surrounded by the growing tissues of the embryo.
- Fate maps of the blastula of chick have been prepared by using the vital stains such as carmine or carbon (charcoal) particles or radioactive thymidine. It shows that blastomeres of area opaca do not form any part of the embryo proper, they form only extra-embryonic membranes.
- Emboly means the throwing in or insertion of cells and epiboly signifies the extending upon. Formation of primitive streak and head process is due to emboly.

6.10 KEY WORDS

- **Coeloblastula**: It is a hollow blastula containing a large spacious blastocoel.
- **Stereoblastula**: It is a type of blastula is composed of an aggregate of larger sized and relatively lesser number of cells without or with extremely small blastocoelic space in the centre.
- **Blastocyst**: It is the blastula stage of mammals; consists of a hollow spherical vesicular blastula, containing an inner cell mass at the animal pole.
- **Concrescence**: It is a term used in older descriptions of gastrulation, denotes the movement of masses of cells toward each other, particularly in the region of the blastopore.

- **Delamination**: It is the splitting or separation of one sheet of cells either into two sheets or separation from other cell groups.
- **Infiltration**: It is a process, where the cells of the blastoderm infiltrate near the bottom of the blastocoel to form a second layer as seen in the gastrulation of chick.

6.11 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short Answer Questions

1. Classify different types of blastula with examples.
2. Explain the process of morphogentics movements with examples.
3. Elaborate the process of gastrulaion in chick.
4. What is mid blastula transition?
5. What do you mean by 'synchrony of cleavage disrupts'.
6. Write a short note on the mechanism of gastrulation in chick.

Long Answer Questions

1. 'The blastulation is the formation of the blastula and blastocoels following the morula stage'. Explain.
2. 'Embryo development begins with a sperm fertilizing an egg to become a zygote which undergoes many cleavage to develop into a ball of cells called a morula'. Write in detail about the process of embryo development.
3. From your learning of the text, discuss about the formation of notochord and mesoderm.
4. 'Gastrulation is an early stage in embryo development in which the blastula reorganizes into the three germ layer: the ectoderm, the mesoderm, and the endoderm'. Discuss gastrulation in frog.
5. 'At least two hours before the bottle cells are formed, internal cells rearrangement occurs which propel the cells from dorsal floor of blastocoel towards animal cap'. from your learning of the text write in detail about the epiboly of prospective ectoderm.

6.12 FURTHER READINGS

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UNIT 7 METABOLIC AND MOLECULAR CHANGES DURING GASTRULATION

Structure

7.0 INTRODUCTION

Gastrulation is a phase early in the embryonic development of most animals, during which the single-layered blastula is reorganized into a multilayered structure known as the gastrula. Before gastrulation, the embryo is a continuous epithelial sheet of cells; by the end of gastrulation, the embryo has begun differentiation to establish distinct cell lineages, set up the basic axes of the body, for example, dorsal-ventral, anterior-posterior, and internalized one or more cell types including the prospective gut. Gastrulation is also the most active phase of embryonic development during which extensive or profound morphogenetic activities of different types of blastomeres occur and the rate of metabolism of gastrula as a whole becomes rapidly increased.

Cells exhibit a wide range of movement. These movements include migration of cells along a surface or through a tissue, or movement of components within cells. Cell motility is one of the crowning achievements of evolution. Primitive cells were probably immobile, carried by currents in the primordial milieu. With the evolution of multicellular organisms, primitive organs were formed by migrations of single cells and groups of cells from distant parts of the embryo. In adult organisms, movements of single cells in search of foreign organisms are integral to the host's defenses against infection; on the other hand, uncontrolled cell migration is an ominous sign of a cancerous cell. Differential cell affinities is a dominant process that drives morphogenesis.

A fate map is a diagram of an egg or blastula, indicating the fate of each cell or region, at a later stage of development. Fate maps are essential tool in most embryological experiments. They provide researchers with information on which portions of the embryo will normally become which larval or adult structure. The analysis of the fate of each blastomere after first and second cleavage is called cytogeny or cell lineage study. Fate map of different types of animals are constructed by multiple methods.

In this unit, you will study about gastrulation, and the metabolic and chemical changes during the phase, catabolism and anabolism and construction of fate maps in detail.

7.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand the metabolic and molecular changes during gastrulation
- Discuss the construction of fate maps
- Learn about cell motility and differential cell affinity
- Explain the different ways of constructing fate maps
- Describe the radioactive isotopes used in constructing fate maps

7.2 METABOLIC AND CHEMICAL CHANGES DURING GASTRULATION

During the process of gastrulation, the late blastula undergoes a striking physiological change in the presumptive organ-forming areas of the epiblast. Interchanges of cells from the epidermal area to the neural area or to the mesodermal area and vice versa are possible during the early phase of gastrulation without disturbing the normal sequence of events. However, such interchanges are not possible at the end of gastrulation. Also, no such physiological changes take place in the hypoblast, i.e., in the presumptive endodermal cells. As the late blastula converses into the late gastrula, the presumptive neural plate ectodermal and epidermal ectodermal areas become changed physiologically, as a result they no longer are determined in a presumptive sense but have undergone changes which make them self-determining. This change is called determination and the biochemical change which effects this alteration is known as chemodifferentiation. As chemo-differentiation involves physiological changes, it restricts changes in potency upon many localized cellular areas. As a result various future organs and parts of organs have their respective fates rigidly and irrevocably determined at the end of gastrulation. Chemo-differentiation apparently occurs through inductive action.

In the molecular aspect of avian development the expression of some embryonic genes begins as early as the morula stage. Spratt (1946), with his

experiment of carbon particles marking in the pre-streak and early -streak stages, had shown that the forward movement of the hypoblast precedes the anterior differentiation of the primitive streak, suggesting that the hypoblast influences the development of the primitive streak on the overlying epiblast. The molecular side of this inducing and axial determining effect of the hypoblast on epiblast is the finding that activin is expressed in the hypoblast and can induce axial structures (notochord, somites and neural tube) in epiblast. The goosecoid gene is expressed in Hensen's node. It is also expressed to some extent in the cells of Koller's sickle, which ultimately forms the Hensen's node. Morphogen, a molecule important in morphogenesis, has also been traced in Hensen's node.

A fate map is a diagram of an egg or blastula, indicating the fate of each cell or region, at a later stage of development. Fate maps are essential tool in most embryological experiments. They provide researchers with information on which portions of the embryo will normally become which larval or adult structure. The analysis of the fate of each blastomere after first and second cleavage is called cytogeny or cell lineage study. The fate map of organisms is helpful in tracing the morphogenetic movements of the cells and the ultimate positions they take up. However, they tell us nothing about the tissue developmental potentialities during morphogenesis.

Motility is the ability of an organism to move independently, using metabolic energy. This is in contrast to mobility, which describes the ability of an object to be moved. Motility is genetically determined but may be affected by environmental factors. For instance, muscles give animals motility but the consumption of hydrogen cyanide (the environmental factor in this case) would adversely affect muscle physiology, causing them to stiffen, leading to rigor mortis. In addition to animal locomotion, most animals are motile (some move by passive locomotion) – the term applies to bacteria and other microorganisms, and to some multicellular organisms, as well as to some mechanisms of fluid flow in multicellular organs and tissue. Motile marine animals are commonly called free-swimming, and motile non-parasitic organisms are called free-living. Metabolism can be studied under following headings:

7.2.1 Catabolism

The morphogenetic movements during gastrulation cause an increased expenditure of energy rich ATP molecules and consequently, an increased oxidation. It has been found that the oxygen consumption during the gastrulation of frog and sea urchin shows a further increase as compared with the blastula stage. During gastrulation the food reserves (for example, glycogen and yolk) are oxidized for the manufacturing of ATP molecules.

- **Oxidation of Glycogen**: One of the substances particularly involved in the supply of energy during gastrulation in amphibians and other animals is glycogen. It has been shown in frogs that amount of glycogen becomes considerably diminished in the invaginating cells of the dorsal lip of the

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blastopore. Rapid breakdown of the glycogen in the dorsal lip suggests a particularly active respiration in this area.

- **Breakdown of Yolk**: Besides glycogen, the yolk of the blastomeres is broken down for energy metabolism or assimilation. The breakdown of yolk granules has been investigated in amphibian embryos both electron microscopically and biochemically. With the electron microscopy, it can be seen that in amphibian embryos, the first change in the yolk platelets consists of the disappearance of the amorphous of granular peripheral layer which contains, besides proteins, considerable quantities of ribonucleic acids. The disappearing material goes into solution in the cytoplasm and becomes available for synthetic processes. The solubilization of the peripheral layer occurs in the invaginating chorda-mesoderm during gastrulation in the neural plate during late gastrulation and early neurulation and still later in the epidermis. The solubilization of the crystalline core (main body) of the yolk platelets occurs considerably later, and in endoderm, it is delayed till just preceding the stage when the larvae start feeding.

7.2.2 Anabolism

Throughout gastrulation, the volume of the embryo does not change appreciably. Every expansion in one direction occurs at the expense of contraction in another direction. Division of cells by mitosis continues throughout gastrulation and this mean, that there is an increase of nuclear material at the expense of the cytoplasmic substances. The anabolic chemical activities of gastrulation includes following important functions:

- **Nucleic Acid Synthesis:** During gastrulation, synthesis of different kinds of nucleic acid molecules occurs. The replication of DNA is needed for the duplication of chromosomes during each mitosis of gastrulation. The onset of gastrulation actually recognized at the molecular level by the start of transcription of DNA dependent rRNA molecules. During gastrulation, the rate of transcription of new mRNA and tRNA molecules become greatly increased which indicates the possibility of increased protein synthesis in the cytoplasm.
- **Protein Synthesis:** During the gastrulation, a sharp increase in protein turns over and in particular protein synthesis takes place. The source of materials for the protein synthesis is mainly the protein yolk, contained in the eggs of most animals. The protein turn over after the beginning of gastrulation seems to be essentially different from what had been going on during cleavage. The new proteins are qualitatively different from those present in the egg. These different kinds of proteins are manufactured in gastrula, only due to active participation of paternal genes in the production of new mRNA molecules during this phase. In sea urchins and amphibians, it has been found that their gastrulae contain antigen proteins, capable of causing the formation of antibodies, which were not present before, (Clayton, 1951).

Throughout gastrulation, the volume of the embryo does not change appreciably. Every expansion in one direction occurs at the expense of a contraction in another direction or directions. What has been said about the absence of growth during cleavage applies in the same way to the period of gastrulation. Division of cells by mitosis continues, however, throughout gastrulation, and thus there is an increase of nuclear material at the expense of the cytoplasmic substances. Breakdown and assimilation of reserve materials are also proceeding, but here a new feature is observed that makes the metabolism of the gastrula different from the metabolism of a blastula.

The morphogenetic movements during gastrulation could be expected to cause an increased expenditure of energy and consequently increased oxidation. This is what is actually found; the oxygen consumption during gastrulation shows a further increase as compared with the cleavage stages and with the blastula. A similar sharp increase in total oxygen consumption is also observed in sea urchin eggs. One of the substances particularly involved in the supply of energy during gastrulation in amphibians is glycogen. It has been discovered that the amount of glycogen becomes considerably diminished in the invaginating cells of the dorsal lip of the blastopore. This was first discovered by histochemical methods, by using a specific stain for glycogen on sections of gastrulating embryos.

Later, by methods of chemical analysis, the exact amounts of glycogen consumed were determined; it was found that in the dorsal lip of the blastopore 31 per cent of the glycogen is lost during gastrulation, whereas in other parts of the embryo only from 1 per cent to 9 per cent is lost during the same time. Rapid breakdown of glycogen in the dorsal lip suggests particularly active respiration in this area. Direct measurements of respiration, however, showed that the dorsal lip region is by no means the part of the gastrula which respires at the highest rate. In order to compare respiration in different parts of the embryo, frog gastrulae were cut into several regions.

Two big pieces were made of the vegetal hemisphere (a dorsal and a ventral one) and four pieces of the marginal zone and the animal hemisphere (pieces 1 to 4), starting with the dorsal lip of the blastopore. The oxygen consumption was determined for each piece as well as the dry weight, total nitrogen and extractable nitrogen, the latter, is equivalent to the nitrogen of the active cytoplasm (total nitrogen less the nitrogen contained in the yolk). When the oxygen consumption is related to dry weight of the fragments, it is seen that there is a great difference in the oxygen consumption of various parts of the embryo. The highest oxygen consumption, as related to dry weight or to total nitrogen that is, to the whole mass of the embryonic tissue is found at the animal pole of the gastrula and the lowest oxygen consumption is at the vegetal pole. The dorsal side, including the dorsal lip, has distinctly higher oxygen consumption than the ventral side.

This would account for the difference in the breakdown of glycogen between the dorsal and the ventral lips. We must realize, however, that not all parts of the cells of a frog gastrula respire; the yolk presumably does not respire at all, whereas

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it contributes to the dry weight and to the total nitrogen of parts of the embryo. It would be desirable to eliminate the yolk from calculations of embryonic respiration, and this was done by calculating the oxygen consumption per unit of extractable nitrogen (nitrogen of active cytoplasm). In other words, the observed differences in oxygen consumption are due to different amounts of active cytoplasm in relation to yolk, that is, to the gradient of yolk distribution and not to a local specifically higher rate. The second peculiarity of the metabolism during the gastrulation period is a sharp increase in protein turnover and particularly in protein synthesis. In a new embryo (Triturus), the rate of synthesis of protein, as measured by the uptake of radioactive precursors, increases five folds between the beginning of gastrulation and the late tail-bud stage when gastrulation is completed and most of the organ rudiments are formed. In the same way, the rate of radioactive precursor intake into proteins in the sea urchin embryo increases roughly three fold between the earliest gastrula, with primary mesenchyme migrating into the blastocoel, and the middle gastrula.

The breakdown of yolk granules has been investigated in amphibian embryos both electrons microscopically and chemically. With the electron microscope, it can be seen that in amphibian embryos the first change in the yolk platelets shows the disappearance of the amorphous or granular peripheral layer which contains, besides protein, considerable quantities of ribonucleic acid. The disappearing material goes into solution in the cytoplasm and becomes available for synthetic processes. The solubilization of the peripheral layer occurs in the invaginating chordomesoderm during gastrulation, in the neural plate during late gastrulation and early neurulation; still later in the epidermis. The solubilization of the crystalline core 'main body' of the yolk platelets occurs considerably later and in endoderm, it is delayed till just preceding the stage when the larvae start feeding.

By both methods it was shown that there is a rapid decrease of yolk platelet protein in the invaginating chordomesoderm starting from the beginning of gastrulation and a slower decrease in the ectoderm in neurulation stages.

In rodents, altered maternal metabolism may have a direct impact on The source of materials for the protein synthesis is mainly the protein yolk, contained in the eggs of most animals.

Chemically, the solubilization of the yolk platelets can be recorded either spectrophotometrically by the decrease of light absorption of the yolk platelets in a microscopic preparation or by separating the yolk platelets from homogenates of embryos and measuring their protein content. Studies have concluded that many congenital malformations are produced during gastrulation and neurulation stages of embryogenesis at a time when no definitive chorioallantoic placenta has been established.

The embryo or an indirect impact via disruption of the nutritive function of the visceral yolk sac. If similar mechanisms operate in human embryos, these factors probably alter functions of the trophoblastic shell. In any case, it is crucial to

remember that the metabolic status of the embryo is rapidly changing and during early stages of organogenesis may respond to alterations in nutrients quite differently during the first four weeks of gestation than at later stages of organogenesis and the fetal period.

7.3 CELL MOTILITY

Cell motility is one of the crowning achievements of evolution. It is a spontaneous movement of a cell from one location to another by consumption of energy. The term encompasses several types of motion, including swimming, crawling, gliding and swarming. Primitive cells were probably immobile, carried by currents in the primordial milieu. With the evolution of multicellular organisms, primitive organs were formed by migrations of single cells and groups of cells from distant parts of the embryo. In adult organisms, movements of single cells in search of foreign organisms are integral to the host's defenses against infection; on the other hand, uncontrolled cell migration is an ominous sign of a cancerous cell. Most cells in the body are stationary, but many of these exhibit dramatic changes in their morphology, the contraction of muscle cells, the elongation of nerve axons, the formation of cell-surface protrusions, the constriction of a dividing cell during mitosis. Perhaps the most subtle movements are those within cells, the active separation of chromosomes, the streaming of cytosol, the transport of memebrane vesicles. These internal movements are essential elements in the growth and differentiation of cells, carefully controlled by the cell to take place at specified times and in particular locations.

All cell movements are a manifestation of mechanical work; they require a fuel (ATP) and proteins that convert the energy stored in ATP into motion, (Refer Figure 7.1). The cytoskeleton, a cytoplasmic system of fibers, is critical to cell motility. Like steel girders supporting the shell of a building, the cytoskeleton plays a structural role by supporting the cell membrane and by forming tracks along which organelles and other elements move in the cytosol. Unlike the passive framework of a building, though, the cytoskeleton undergoes constant rearrangement, which can produce movements.

In the electron microscope, the cytoskeleton appears as a dense and seemingly random array of fibers. However, we now recognize that this array consists of three types of cytosolic fibers: microfilaments, 7 - 9nm in diameter; intermediate filaments, 10nm in diameter; and microtubules, 24nm in diameter. These cytoskeletal fibers are well-ordered polymers built from small protein subunits held together by non-covalent bonds. Instead of being a disordered array, the cytoskeleton is organized into discrete structures, primarily bundles, geodesic-dome-like networks and gel-like lattices. Although the primary function of intermediate filaments is structural, to reinforce cells and to organize them into tissues, because microtubules and intermediate filaments are often associated with one another.

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Cells have evolved two basic mechanisms for generating movement. One mechanism involves a special class of enzymes called motor proteins. These proteins use energy from ATP to walk or slide along a microfilament or a microtubule. Some motor proteins carry membrane-bound organelles and vesicles along the cytoskeletal fiber tracks; other motor proteins cause the fibers to slide past each other. The other mechanism responsible for many of the changes in the shape of a cell entails assembly and disassembly of microfilaments and microtubules. A few movements involve both the action of motor proteins and cytoskeleton rearrangements. It is essential to a variety of biological processes such as the development of an organism (morphogenesis), wound healing, cancer metastasis and immune response. For example, during morphogenesis there is a targeted movement of dividing cells to specific sites to form tissues and organs. For wound healing to occur, cells such as neutrophils (white blood cells) and macrophages (cells that ingest bacteria) move to the wound site to kill the microorganisms that cause infection, and fibroblasts (connective tissue cells) move there to remodel damaged structures. In all these examples, cells reach their target by crawling. There are also other kinds of motility, such as the swimming of most sperm cells, and the movement of some bacteria by the rotation of flagellar motors. Cell crawling, however, is the common mechanism employed by most motile eukaryotic animal cells.

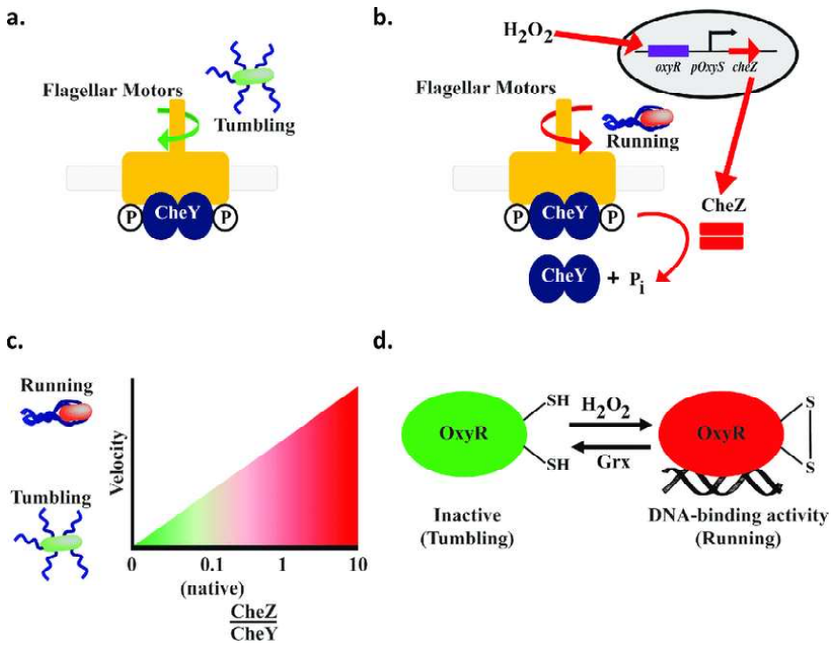

Fig. 7.1 *Hydrogen Peroxide Controlled Bacterial Motility*

Cell movement was observed as early as 1675 when van Leeuwenhoek saw cells crawl across his microscope slide, the molecular mechanisms behind cell movement have become a scientific focus only in the past few decades. Advances in fluorescence microscopy, molecular biology and biochemistry have enabled the

discovery of the processes underlying motility and the identification of the major proteins behind these processes. These experimental techniques alone, however, cannot adequately explain whether these proteins are capable of generating the required forces for motility nor the physical mechanisms employed. A significant advance in this direction was made when biophysical studies helped identify regions where different force generating proteins are located, measured (*in-vitro*) the exact forces generated by some of these proteins and measured (*in-vivo*) the forces associated with movement.

As a cell moves on a substrate (the extracellular matrix if the cell moves inside an organism or a cover slide if it moves outside an organism), it experiences external forces, which include the viscous force or resistance from the surrounding medium and cell-substrate interaction forces, and internal forces that are generated by the cytoskeleton. In most animal cells, the cytoskeleton is the essential component in creating these motility-driving forces and in coordinating the entire process of movement. The cytoskeleton is a polymer network, composed of three distinct biopolymer types: actin, microtubules and intermediate filaments. These biopolymers are differentiated principally by their rigidity which can be described by the persistence length L_p. The persistence length is defined as the distance over which the filament is bent by thermal forces and increases with increasing fitness.

These movements include migration of cells along a surface or through a tissue or movement of components within cells. Specific examples of cell motility include:

- Movement of cells from one location in an embryo to another during embryonic development.
- Migration of cells into a wound during wound healing.
- Contraction of a muscle cells that is the fundamental process responsible for muscle contraction.
- Separation of a cell into two daughter cells during cell division
- Movement of membrane-bound vesicles into cells during phagocytosis or endocytosis.
- Movement of membrane-bound vesicle from the cell interior to the cell surface during secretion.
- movement of chromosomes during mitosis.

The first four bulleted points are examples of cell movement, while the last three bulleted points are examples of 'intracellular motility'. All of these movements have in common the fact that they are mediated by filamentous structures in the cell called the cytoskeleton and are powered by molecular motors that move along these filamentous structures. The simplest example is the movement of membrane-bound vesicles. These vesicles bind to a molecular motor just like a boxcar attaches to a railroad locomotive. The vesicle represents the cargo and the molecular motor represents the locomotive. The molecular motor then moves along the cytoskeletal

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filament as a locomotive moves along a railroad track. Most forms of intracellular movement occur using this mechanism.

A second mechanism is called contraction. This mechanism is responsible for contraction of muscle cells and the separation of daughter cells during cell division. Contraction works through the action of molecular motors pulling on the cytoskeletal filaments, drawing them toward each other. A third mechanism involves the rapid polyermization of the cytoskeleton. In this case the filamentous structures (usually the microfilament cytoskeleton) extend by the addition of subunits to the end. This growth of filaments then pushes out the membrane. This mechanism is responsible for protrusion of the front end of migrating cells. Moving cells exhibit a special kind of directional movement called **chemotaxis**. This mechanism accounts for the ability of cells to migrate in a specific direction. During chemotaxis, cells move in response to an external signal, most frequently a small molecule or short peptide, called a chemoattractant. Cells sense the concentration of the chemical and move in the direction of increasing concentration of the signal. This directional movement is responsible for much of the cell migration required for tissue formation and for wound healing. Wounded cells, for instance, release chemoattractants that attract immune system cells called macrophages and connective tissue cells called fibroblast.

For most eukaryotic cells, the process of cell movement occurs in several coordinated steps. First, cells extend a structure called as pseudopod (false foot) using the polymerization mechanism. Next the pseudopod makes an attachment to the surface along which the cell is moving. This establishes the new front of the cell. A contraction-mediated process provides the force that moves the rest of the cell toward the front and leads to detachment of the trailing end of the cell. When cells are exhibiting chemotaxis they extend pseufopods in several directions, but only make the attachment to the surface in the direction of the highest concentration of the chemical signal. In contrast, bacterial cells move by the action of an amazing rotary motor called a bacterial flagellum. This flagellum spines like a propeller propelling the cell forward. Bacteria undergo chemotaxis by a process called 'tumble and run'. The motor proteins that move along microtubules. Kinesins move toward the plus end, whereas dyneins move toward the minus end.

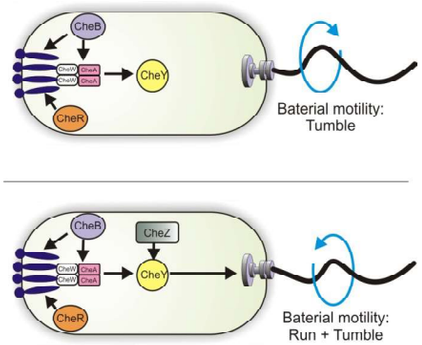

***Fig. 7.2** Bacterial Motility*

In this process, a bacterial cell tumbles end over end and then 'runs', moving in a single (random) direction for a defined period of time, (Refer Figure 7.2). At the end of that period the cell stops, tumbles again and measures the concentration of the chemoattractant. If the concentration of chemoattractant is higher than at the last sampling the cell runs for an increased distance. If the chemoattractant concentration is lower, the run distance is shortened and the cell tumbles more frequently. Through this biased process cells preferentially migrate in the direction of higher chemoattractants.

Check Your Progress

1. What are differential cell affinities?
2. What happens during the process of gastrulation?
3. What happens with the evolution of multicellular organisms?
4. Name a crowning achievement of evolution.

7.4 DIFFERENTIAL CELL AFFINITY

Undoubtedly it's a matter of deep discussion that how different scattered and separate populations of cells forms different types of cells; then how newly formed tissues unite to form different organs and so formed organs acquires specific position in the fetus. Not only this much but how do different cells of the embryo grows throughout the course of embryonic development? And answers to all these questions can be understood after the concept of differential cell affinity. All the cells present in the embryo are either epithelial cells or mesenchymal cells in which the epithelial cells can form tubes and other cells remain intact to each other whereas mesenchymal cells frequently migrates individually and form extracellular matrices that helps in keeping the blastomeres altogether. The cell surface looks pretty much the same in all cell types and many early investigators thought that the cell surface was not even a living part of the cell. Every type of cell has a different set of proteins in its surfaces and that some of these differences are responsible for forming the structure of the tissues and organs during development.

Scientific studies by Holtfreter described the term 'selective affinity'. The inner surface of the ectoderm has a positive affinity for mesodermal cells and a negative affinity for the endoderm, while the mesoderm has positive affinities for both ectodermal and endodermal cells. Mimicry of normal embryonic structure by cell aggregates is also seen in the recombination of epidermis and neural plate cells. The presumptive epidermal cells migrate to the periphery as before; the neural plate cells migrate inward, forming a structure reminiscent of the neural tube. When axial mesoderm (notochord) cells are added to a suspension of presumptive epidermal and presumptive neural cells, cell segregation results in an external epidermal layer, a centrally located neural tissue and a layer of mesodermal

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tissue between them. Somehow, the cells are able to sort out into their proper embryonic positions.

Such selective affinities were also noted by Boucaut (1974), who injected individual cells from specific germ layers into the body cavity of amphibian gastrulae. He found that these cells migrated back to their appropriate germ layer. Endodermal cells found positions in the host endoderm, whereas ectodermal cells were found only in host ectoderm. Thus, selective affinity appears to be important for imparting positional information to embryonic cells. Embryonic cells do not retain a single stable relationship with other cell types. For development to occur, cells must interact differently with other cell populations at specific times. Such changes in cell affinity are extremely important in the processes of morphogenesis.

Recent studies showed that boundaries between tissues can indeed be created both by different cell types having different types of cell adhesion molecules and different cell types having different amounts of cell adhesion molecules. There are several classes of molecules that can mediate cell adhesion. The major cell adhesion molecules appear to be the cadherins. As their name suggests, they are calcium-dependent adhesion molecules. Cadherins are critical for establishing and maintaining intercellular connections and they appear to be crucial to the spatial segregation of cell types and to the organization of animal form (Takeichi 1987). Cadherins interact with other cadherins on adjacent cells and they are anchored into the cell by a complex of proteins called catenins. The cadherin-catenin complex forms the classic adherens junctions that connect epithelial cells together. Moreover, since the catenins bind to the actin cytoskeleton of the cell, they integrate the epithelial cells together into a mechanical unit. In vertebrate embryos, several major cadherin classes have been identified:

- **E-Cadherin** (epithelial cadherin, also called uvomorulin and L-CAM) is expressed on all early mammalian embryonic cells, even at the 1-cell stage. Later, this molecule is restricted to epithelial tissues of embryos and adults.
- **P-Cadherin** (placental cadherin) appears to be expressed primarily on the trophoblast cells (those placental cells of the mammalian embryo that contact the uterine wall) and on the uterine wall epithelium (Nose and Takeichi 1986). It is possible that P-cadherin facilitates the connection of the embryo to the uterus, since P-cadherin on the uterine cells is seen to contact P-cadherin on the trophoblast cells of mouse embryos (Kadokawa et al. 1989).
- **N-Cadherin** (neural cadherin) is first seen on mesodermal cells in the gastrulating embryo as they lose their E-cadherin expression. It is also highly expressed on the cells of the developing central nervous system; Hatta and Takeichi 1986).
- **EP-Cadherin** (C-cadherin) has been found to be critical for maintaining adhesion between the blastomeres of the Xenopus blastula and is required for the normal movements of gastrulation.

- **Protocadherins** are calcium-dependent adhesion proteins that differ from the classic cadherins in that they lack connections to the cytoskeleton through catenins. Protocadherins have been found to be very important in separating the notochord from the other mesodermal tissues during Xenopus gastrulation.

Cadherins join cells together by binding to the same type of cadherin on another cell. Thus, cells with E-cadherin stick best to other cells with E-cadherin, and they will sort out from cells containing N-cadherin in their membranes, (Refer Figure 7.3). This pattern is called homophilic binding. Cells expressing N-cadherin readily sort out from N-cadherin-negative cells in vitro and univalent (Fab) antibodies against cadherins will convert a three-dimensional, histotypic aggregate of cells into a single layer of cells.

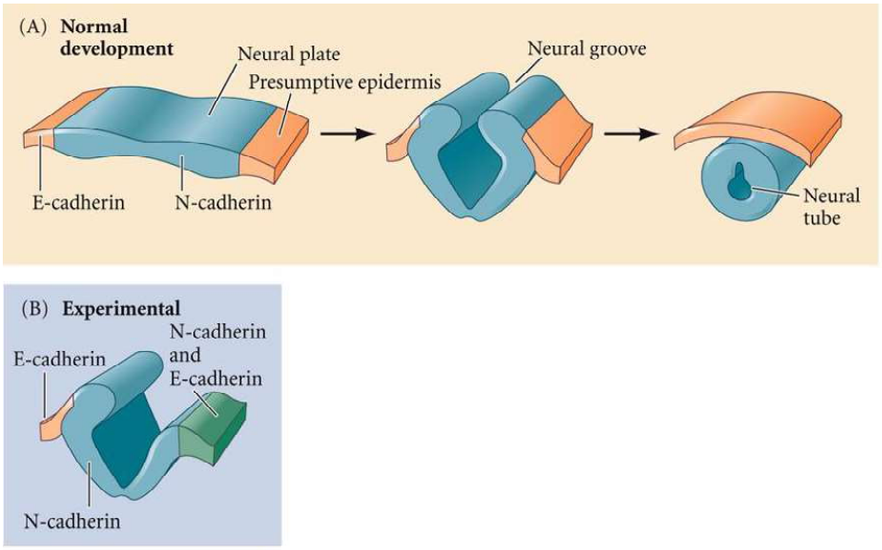

Fig. 7.3 *Expression of N- and E-Cadherin Adhesion Proteins during Neurulation in* Xenopus

This is how during the development, different cells remain intact during development and acquires the ability to perform specific functions which accounts for the differential ability.

7.5 FATE MAP CONSTRUCTION

A fate map is a diagram of an egg or blastula, indicating the fate of each cell or region, at a later stage of development. Fate maps are essential tool in most embryological experiments. They provide researchers with information on which portions of the embryo will normally become which larval or adult structure. The analysis of the fate of each blastomere after first and second cleavage is called cytogeny or cell lineage study. It describes what it will become in the course of normal development. The fate of a particular cell can be discovered by labelling

that cell and observing what structures it becomes a part of. When the fate of all cells of an embryo has been discovered, we can build a fate map, which is a diagram of that organism at an early stage of development that indicates the fate of each cell or region at a later stage of development. A fate map tells us which parts of the egg or early embryo contributes to specific tissues or structures at some later, advanced stage of development. Some animals may have a very strict fate map, in which particular parts of the egg or cleavage stage blastomeres always contribute to particular parts of the larva or adult. Examples include the nematode *C. elegans*, or the urochordate tunicates (sea squirts) where as some animals do not have a very predictable fate map (such as mammals) or other animals have fate maps.

Construction of Fate Map

Various techniques have been devised for the construction of fate map. Of these tracing the course of natural colours and artificial markings are most important. In practice one makes some sort of 'mark' on, or inside, the egg or embryo, with any number of agents: charcoal, dye, soot (old fashioned ways), or modern agents, like fluorescent molecules (rhodamine conjugated dextran) or proteins like Green Fluorescent Protein (GFP), or enzymes encoded by injected genes or mRNAs. Whichever way you choose, the principal is the same: Make your mark at some time '0' and score where the mark is at a later time(s) 'x'. It is also important to be able to orient your marks on an embryo with respect to some asymmetric feature, such as a pigment difference or a unique structure:

- **Natural Markings:** The cytoplasm of certain eggs such as those of ascidians has natural pigments. Thus in eggs of Styela four coloured centres have been recognized, an upper hemisphere of light protoplasm, a yellow crescent postereo-ventrally, a grey crescent antero-dorsally and a vegetal area of dark grey yolky substance. The fate of these areas can be followed very easily. It has been revealed that the upper clear cytoplasm contains the material for epidermal ectoderm. The grey crescent area differentiates into the prospective neurectodem and notochord. The yellow crescent becomes the prospective mesoderm and the dark grey yolky area forms prospective endodermal structure
- **Artificial Markings:** There are three methods to mark or label the early blastomeres by which their fate can be traced out. They are vital staining. Early embryologists used 'vital dyes' (which would stain but not harm the cells) to follow movements of individual cells or groups of cells. The tissues to which the cells contribute would thus be labeled and visible in the adult organism. The first person to develop and use this technique to study cell fate was embryologist Walter Vogt in 1929. Vogt used small chips of agar impregnated with a vital dye, (such as Nile Blue or Nile Red) which he placed on a particular cell or population of cells in *Xenopus embryos* until the dye absorbed into the yolk platelets within the desired cells. Once the

cells were effectively labeled, the agar chip could be removed and the embryo was allowed to develop normally. With this method, Vogt was able to distinguish movements of particular cell populations and the ultimate organ or tissue into which they integrated. Vital stains are mild blue, sulphate, neutral fed, Jenus green ,etc.

- **Carbon Particle Marking**: This technique was introduced by Spratt (1946) to demonstrate the process involved in primitive streak formation in chick. This consists of applying tiny particles of carbon over the surface of blastomeres. They stick to the cell surface and enable to follow the movements of the cells and to determine the fate of these blastomeres.
- **Radioactive Isotope Labeling**: The radioactive isotope such as C^{14} and P are used to label the early blastomeres. By carefully following the course of these radioactive isotopes the fate of blastomeres can be determined.

Fate Map of Typical Chordate Blastula

The following presumptive areas are discernible in chordate blastula:

- There is a broad ectodermal area in the animal hemisphere which forms the epidermal layer of the skin. This is known as epidermal ectoderm.
- A relatively smaller ectodermal area lies below the epidermal ectoderm. This area is known as neurectoderm since it contributes to the formation of neural tube and nervous system.
- A crescentic area below the neurectoderm is designated as notochordal area which gives rise to the notochord of the embryo.
- On either side of the notochordal area are two lateral areas. These constitute the prospective mesoderm.
- Most of the yolky vegetal hemisphere blastomeres collectively form the prospective endoderm.
- At the caudal margin of the notochordal area is a small strip of blastomeres called prechordal plate region. This region mainly gives rise to some of the head mesoderm.

Methods for Fate Map of Typical Chordate Blastula

Fate map of different types of animals have been constructed by the following methods:

- **Observing Living Embryos:** In some invertebrates, the embryos being transparent and having relatively few daughter cells that remain close to one another, it has been possible to look through the microscope and trace the descendants of a particular cell to the organ they subsequently form. This type of study was performed by Edwin G. Conklin (1905) in the tunicate, Styela partita, where the different cells contain different pigments. As for example, the muscle-forming cells always have a yellow color.

- **Vital Dye Marking:** Most embryos, however, do not have the facilities (transparent, few cells, different colours, etc.) as described above in Styela partita. It was in 1929 that Vogt was able to trace the fate of different areas of amphibian eggs by applying vital dyes. These vital dyes stain the cells without killing them.
- **Radioactive Labelling and Fluorescent Dyes:** A variation of the dye marking technique is to make one area of the embryo radioactive. A donor embryo is taken and grown in a solution containing radioactive thymidine. This thymidine base is subsequently incorporated into the DNA of the dividing embryo. A second embryo, acting as the host embryo, is grown under normal conditions. The region of interest is cut off from the host embryo and is replaced by a radioactive graft from the donor embryo. The cells that are radioactive will be the descendants of the cells of the graft and are distinguished by autoradiography.
- **Genetic Marking:** Radioactive and vital dye marking have their own drawbacks such as dilution over many cell divisions and the laborious preparation of slides. One permanent way of cell marking is to create mosaic embryos having different genetic constitutions. The best example of such a marking is to graft quail cells inside a chick embryo. By doing so, fine-structure maps of the chick brain and skeletal system can be made.

Fate map of *Amphioxus*

The fate map of Amphioxus can be traced at an early stage prior to the onset of cleavage. The presumptive organ forming areas in the uncleaved egg is given in Figure 7.4. The future endodermal cells lie at the vegetal pole and would subsequently form the floor or hypoblast of the blastula. The area at the animal pole would form the presumptive ectodermal cells.

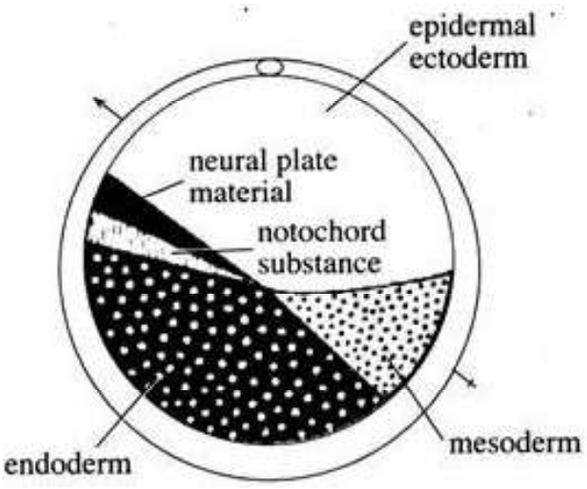

Fig. 7.4 *Fate Map of Uncleaved Egg of Amphioxus showing Presumptive Organ Forming Areas*

The ventral grey crescent area at the future posterior end of the blastula, in between the future ectoderm and endoderm, forms the future mesoderm. Another area, the dorsal crescent, in between the ectoderm and endoderm on the anterior side, gives rise to the notochord and neural cells. The presumptive ectodermal, mesodermal, notochordal and neural cells would subsequently form the epiblast of the blastula.

Fate Map of Frog

The blastula of *Xenopus* at the 32 cell stage gives no indication as to how the different regions will develop. However, by following the fate of individual cell, or group of cells, the fate map of the blastula can be made. One way of making the fate map is by staining the various parts of the early embryo with a lipophilic dye such as dil and observe where the labelled regions end up. Another sophisticated way of labelling the blastomeres is by injection of high molecular weight molecules such as rhodamine-labelled dextran, which cannot pass through cell membrane and are, therefore, restricted to the injected cell and its progeny. These cells can be easily detected later, under a UV microscope.

The fate map of the Xenopus blastula (Refer Figure 7.5) shows the presence of yolky macromeres at the vegetal pole which gives rise to the endoderm. Depending upon the position of the blastopore, the endodermal area can be divided into the sub-blastoporal and supra-blastoporal endoderm. The cells towards the animal pole give rise to the ectoderm, which becomes further subdivided into epidermis and the future nervous tissue. The epidermal ectoderm forms at the ventral side of the animal hemisphere, while the neural ectoderm forms at the dorsal side. The mesoderm forms a belt-like region, known as the marginal zone, around the equator of the blastula.

The mesoderm becomes subdivided along the dorsoventral axis of the blastula. The most dorsal mesoderm gives rise to the notochord. From this; ventrally, the- mesoderm is differentiated by the somites (which gives rise to muscle tissue), lateral plate (which contains heart and kidney mesoderm) and blood islands. In *Xenopus*, a thin outer layer of presumptive endoderm overlies the presumptive mesoderm in the marginal zone.

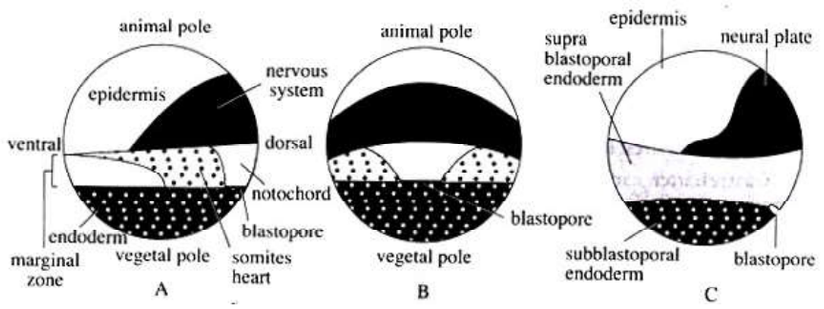

Fig. 7.5 *Fate Map of Frog Egg: Late Blastula (A); Dorsal View (B); Exterior View (c) Showing Presumptive Organ forming Areas*

Fate Map of Chick

Before going through the fate map of chick one should go through the formation of area pellucida and area opaca and also through the formation of hypoblast and epiblast, (Refer Figure 7.6). From the study of the above formations, it becomes clear that the hypoblast does not contribute any cells to the formation of the embryo proper, rather they contribute to the formation of a portion of the external membranes. Recent studies with Cell Adhesion Molecules (CAMs), has made possible to construct the fate map of chick epiblast. All the three germ layers of the embryo proper are formed by the epiblastic cells. The epiblast also forms a considerable amount of extra-embryonic (mesoderm) membrane.

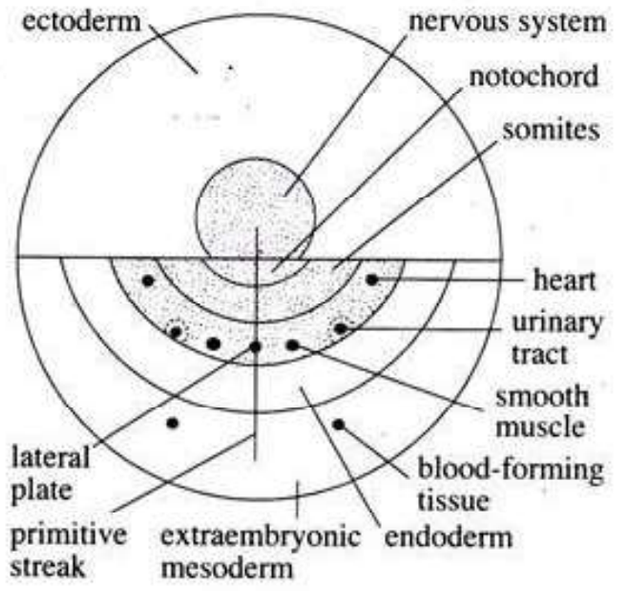

Fig. 7.6 *Fate Map of Chick Embryo showing Presumptive Organ forming Areas*

The fate map of chick reveals that the cells of the epiblast are organised around the notochord and nervous system. The neural ectoderm is present as a knob-like structure facing towards the anterior side. The cells at the anterior part of the epiblast form the ectoderm, while the cells at the posterior side gives rise to mesoderm (body proper), endoderm and extra-embryonic mesoderm.

Usefulness of Fate Map

The fate map of organisms is helpful in tracing the morphogenetic movements of the cells and the ultimate positions they take up. However, they tell us nothing about the tissue developmental potentialities during morphogenesis. Creating a fate map is a valuable part of understanding an organism's developmental pathway. Understanding the lineage and migration of progenitor cells can lead to the discovery of gene regulatory networks and signaling pathways. Furthermore, determining

the structural make up of an organism can possibly lead to determining the function of each specific region. The possibility of new developmental discoveries comes with the creation of each new fate map.

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Check Your Progress

5. What is a fate map?
6. How can the fate map of Amphioxus be traced?
7. What forms the future mesoderm?
8. What is the fate map of organisms helpful in?

7.6 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Differential cell affinities is a dominant process that drives morphogenesis.
2. During the process of gastrulation, the late blastula undergoes a striking physiological change in the presumptive organ-forming areas of the epiblast.
3. With the evolution of multicellular organisms, primitive organs were formed by migrations of single cells and groups of cells from distant parts of the embryo.
4. Cell motility is one of the crowning achievements of evolution.
5. A fate map is a diagram of an egg or blastula, indicating the fate of each cell or region, at a later stage of development.
6. The fate map of Amphioxus can be traced at an early stage prior to the onset of cleavage.
7. The ventral grey crescent area at the future posterior end of the blastula, in between the future ectoderm and endoderm, forms the future mesoderm.
8. The fate map of organisms is helpful in tracing the morphogenetic movements of the cells and the ultimate positions they take up.

7.7 SUMMARY

- Gastrulation is a crucial step in early embryogenesis. During gastrulation, a set of morphogenetic processes takes place leading to the establishment of the basic body plan and formation of primary germ layers.
- Differential cell affinities is a dominant process that drives morphogenesis. Using an alkaline solution, they made single cell suspensions from the ectoderm, endoderm and mesoderm layers of an embryo soon after neural tube formation.

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- During the process of gastrulation, the late blastula undergoes a striking physiological change in the presumptive organ-forming areas of the epiblast.
- As chemo-differentiation involves physiological changes, it restricts changes in potency upon many localized cellular areas.
- A fate map is a diagram of an egg or blastula, indicating the fate of each cell or region, at a later stage of development. Fate maps are essential tool in most embryological experiments.
- Chemically, the solubilization of the yolk platelets can be recorded either spectrophotometrically by the decrease of light absorption of the yolk platelets in a microscopic preparation or by separating the yolk platelets from homogenates of embryos and measuring their protein content.
- Cell motility is one of the crowning achievements of evolution. It is a spontaneous movement of a cell from one location to another by consumption of energy.
- With the evolution of multicellular organisms, primitive organs were formed by migrations of single cells and groups of cells from distant parts of the embryo.
- In adult organisms, movements of single cells in search of foreign organisms are integral to the host's defenses against infection; on the other hand, uncontrolled cell migration is an ominous sign of a cancerous cell.
- In the electron microscope, the cytoskeleton appears as a dense and seemingly random array of fibers.
- All the cells present in the embryo are either epithelial cells or mesenchymal cells.
- The cell surface looks pretty much the same in all cell types and many early investigators thought that the cell surface was not even a living part of the cell.
- Every type of cell has a different set of proteins in its surfaces and that some of these differences are responsible for forming the structure of the tissues and organs during development.
- Recent studies showed that boundaries between tissues can indeed be created both by different cell types having different types of cell adhesion molecules and different cell types having different amounts of cell adhesion molecules.
- Cadherins join cells together by binding to the same type of cadherin on another cell. Thus, cells with E-cadherin stick best to other cells with E-cadherin, and they will sort out from cells containing N-cadherin in their membranes.
- A fate map is a diagram of an egg or blastula, indicating the fate of each cell or region, at a later stage of development.
- The analysis of the fate of each blastomere after first and second cleavage is called cytogeny or cell lineage study.

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- Some animals may have a very strict fate map, in which particular parts of the egg or cleavage stage blastomeres always contribute to particular parts of the larva or adult.
- Various techniques have been devised for the construction of fate map. Of these tracing the course of natural colours and artificial markings are most important.
- The blastula of Xenopus at the 32 cell stage gives no indication as to how the different regions will develop.
- The mesoderm becomes subdivided along the dorsoventral axis of the blastula. The most dorsal mesoderm gives rise to the notochord.
- The fate map of chick reveals that the cells of the epiblast are organised around the notochord and nervous system.
- The fate map of organisms is helpful in tracing the morphogenetic movements of the cells and the ultimate positions they take up.
- The molecular side of this inducing and axial determining effect of the hypoblast on epiblast is the finding that activin is expressed in the hypoblast and can induce axial structures (notochord, somites and neural tube) in epiblast. The goosecoid gene is expressed in Hensen's node.
- A fate map is a diagram of an egg or blastula, indicating the fate of each cell or region, at a later stage of development. Fate maps are essential tool in most embryological experiments. They provide researchers with information on which portions of the embryo will normally become which larval or adult structure.
- The analysis of the fate of each blastomere after first and second cleavage is called cytogeny or cell lineage study. The fate map of organisms is helpful in tracing the morphogenetic movements of the cells and the ultimate positions they take up.
- Motility is the ability of an organism to move independently, using metabolic energy. This is in contrast to mobility, which describes the ability of an object to be moved. Motility is genetically determined but may be affected by environmental factors.
- The bacterial cells move by the action of an amazing rotary motor called a bacterial flagellum. This flagellum spins like a propeller propelling the cell forward. Bacteria undergo chemotaxis by a process called "tumble and run". The motor proteins that move along microtubules. Kinesins move toward the plus end, whereas dyneins move toward the minus end.
- All the cells present in the embryo are either epithelial cells or mesenchymal cells in which the epithelial cells can form tubes and other cells remain intact to each other whereas mesenchymal cells frequently migrates individually and form extracellular matrices that helps in keeping the blastomeres altogether.

- The cell surface looks pretty much the same in all cell types and many early investigators thought that the cell surface was not even a living part of the cell. Every type of cell has a different set of proteins in its surfaces and that some of these differences are responsible for forming the structure of the tissues and organs during development.

7.8 KEY WORDS

- **Cell motility:** It is one of the crowning achievements of evolution. It is a spontaneous movement of a cell from one location to another by consumption of energy. The term encompasses several types of motion, including swimming, crawling, gliding and swarming.
- **Carbon particle marking**: It is technique introduced by Spratt (1946) to demonstrate the process involved in primitive streak formation in chick. This consists of applying tiny particles of carbon over the surface of blastomeres. They stick to the cell surface and enable to follow the movements of the cells and to determine the fate of these blastomeres.
- **Radioactive isotope labeling**: This is a kind of labeling in which the radioactive isotope such as C^{14} and P are used to label the early blastomeres.
- **Artificial markings:** It is a marking method to mark or label the early blastomeres by which their fate can be traced out.

7.9 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short Answer Questions

1. Name the radioactive isotopes use in constructing fate maps.
2. In chordate blastula, which area gives rise to notochord of the embryo?
3. Who developed the technique of vital staining used for constructing fate maps?
4. Define fate maps.
5. Who described the term Selective Affinity for the first time?

Long Answer Questions

1. Describe differential cell affinity in detail.
2. Diagrammatically explain the fate map of chick embryo.
3. Explain the different ways of constructing fate maps.
4. Enlist the metabolic and chemical changes occurring during gastrulation.
5. Draw and explain the fate map of frog embryo.

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BLOCK - III
ORGANOGENESIS AND ASSISTED
REPRODUCTIVE TECHNOLOGY

UNIT 8 EMBRYONIC DEVELOPMENT STAGES OF CHICK

Structure

8.0 INTRODUCTION

Vertebrate eye development begins with the formation of the optic vesicles as outgrowths of the forebrain. These initial pouches grow laterally and can be subdivided into optic stalk and optic vesicle. The axis of growth then shifts to produce optic vesicles that enlarge dorsally to lie alongside the expanding diencephalon. Concomitant invagination of the optic vesicles and the overlying ectoderm produces the optic cup and lens. During later stages, the lens detaches from the surfaces ectoderm and the optic cup forms the neural retina and the pigmented epithelium. Experimental analysis of eye development has revealed an intimate relationship between invagination of the lens and optic cup. The primordia of the lens and neural retina become adherent, as a result of changes in the extracellular matrix, before invagination commences. Interference with matrix synthesis causes abnormal development of the optic cup, and subsequent abnormalities of the lens.

The neural tube formed from the neural ectoderm is located dorsally in the median plain of the embryo and forms the basis for the central nervous system.

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Before going into the development of brain a brief idea about the formation of neural tube in chick is dealt with. During gastrulation of chick a primitive streak with Hensen's node is formed. In the 24 hours chick-embryo, as epiboly of ectodermal cells is taking place at the surface of the embryo, it brings about an elongation of the presumptive neural ectodermal cells above the notochordal area. Two types of heart are present in the vertebrate groups – lymph hearts and the heart of the arteriovenous system. The latter one is a centralized, well-muscularized organ, present ventral to the oesophageal segment of the gut.

In this unit you will study about the development process of the eye, brain, heart, muscles, nervous system in chick, embryonic induction and the organizer concept in detail.

8.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand the development of eye, brain and heart in chick
- Know the formation of muscles and nervous system in chick
- Learn about embryonic induction and organizer concept
- Discuss the structure of the eye and lens in a four-day chick
- Explain mechanism of embryonic induction in different types of gastrula
- Discuss the role of organizer during development of an embryo
- Describe the development of eye in chick

8.2 DEVELOPMENT OF EYE IN CHICK

The major development of the eye involves ectoderm, neural crest cells, and mesenchyme. The neural tube ectoderm gives rise to the retina, the iris and ciliary body epithelia, the optic nerve, the smooth muscles of the iris, and some of the vitreous humor. Surface ectoderm gives rise to the lens, the conjuctival and corneal epithelia, the eyelids, and the lacrimal apparatus. The remaining ocular structures develop from mesenchyme, (Refer Figure 8.1).

The two small grooves develop on each side of the developing forebrain in the neural folds called optic grooves or optic sulci. As the neural tube closes, these grooves become out-pocketings and are now called optic vesicles grow toward the ectoderm, their connections to the forebrain become attenuated to form optic stalks which will eventually become the optic nerves. The portion of each optic vesicle that interacts with the surface ectoderm induces that area of the ectoderm to form a thickening called the lens placode (a precursor of the lens) that invaginates to become a lens pit and finally lens vesicle. At the same time the lens vesicle is forming, the optic vesicle also invaginates to form a double-layered structure called

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the optic cup. The two layers of the optic cup will further differentiate into the retina of the mature eye. The eyelids begin to form from neural crest cells as well as surface ectoderm just anterior to the cornea.

The neural tube formed from the neural ectoderm is located dorsally in the median plain of the embryo and forms the basis for the central nervous system. During gastrulation of chick a primitive streak with Hensen's node is formed. The epiboly of ectodermal cells of chick-embryo, brings about an elongation of the presumptive neural ectodermal cells above the notochordal area. As gastrulation comes to an end, the single-layered neural ectoderm rapidly becomes thick and stratified to form the neural plate. The neural plate gradually sinks down and its margins become elevated resulting in the formation of a neural groove bounded by the neural folds that in turn meet to form the neural tube. The primordial brain tube is initially divided into an anterior prosencephalon (archencephalon) and posterior deuteroencephalon. The prosencephalon is induced by prechordal plate mesoderm and the deuteroencephalon by the anterior portion of the notochordal mesoderm, both lying beneath the primordial brain.

The heart is among the earliest, largest, and most easily studied organs to form during embryogenesis. In its earliest stages (during gastrulation), cardiogenic mesoderm, which had entered the primitive streak now migrates from the lateral portions of the embryo back towards the midline near the pharyngeal region. There it forms two parallel tubes, one on either side of the midline, that soon fuse into one. Mesodermal components of this tube will form the inner endocardial layer of the heart, the muscular myocardium, and the outer pericardium, as well as the septa and valves of the embryonic and adult hearts. From anterior to posterior, these include: the truncus arteriosus (conus artereriosus; conotruncus), ventricle, atrium, and the sinus venosus, which splits posteriorly to join with the vitelline veins. Contractile activity appears to be an inherent function of individual cells, and coordinated beating appears in the chick in the unpaired heart primordial tubes just prior to fusion. As the heart forms, it beats in rhythmic waves, which are coordinated electrophysiologically by cells in the sinus venosus. At this stage of heart development, this wavelike motion moves blood from the posterior to the anterior of the heart.

Embryonic induction describes the embryonic process in which one group of cells, the inducing tissue, directs the development of another group of cells, the responding tissue. Induction directs the development of various tissues and organs in most animal embryos; for example, the eye lens and the heart. The effect of embryonic interaction or organizer is a morphogenetic effect by which one organic tissue transmits a chemical substance that influences other embryonic part to produce a structure that otherwise could not come into existence. The embryonic tissue which exerts such an influence is called an inductor and the chemical substance secreted by an inductor is known as evocators. The tissue on which evocator works and the tissue responses is known as responsive tissue. The action of the indicator through evocator is known as induction action or organizer action.

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This process of induction influences greatly the protein synthesis mechanism of responsive tissues as a result of which definite structure forming cells become very active.

The primary optic vesicles arise in chicks of about 30 hours as dilations in the lateral wall of the prosencephalon. At first the optic vesicles open broadly into the brain, but later constrictions develop which narrow their attachment to form a stalk. In chicks of 55 hours the primary optic vesicles are invaginated to form the double-walled secondary optic vesicles or optic cups. The invagination takes place in such a way that the ventral wall of the cup is incomplete; the gap in it being known as the choroid fissure. The lens arises as a thickening of the superficial ectoderm which becomes depressed to form a vesicular invagination extending into the optic cup.

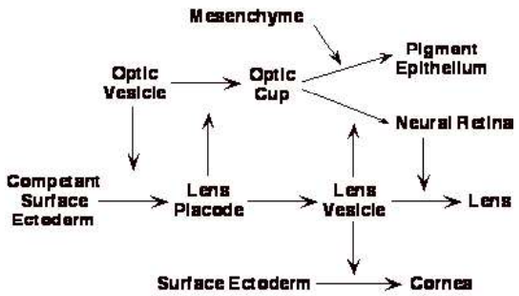

Fig. 8.1 *Tissue Interactions during Eye Development*

In chicks of four days the choroid fissure has become narrowed by the growth of the walls of the optic cup on either side of it. The orifice of the optic cup becomes narrowed by convergence of its margins toward the lens. Meanwhile the lens has become free from the superficial ectoderm and forms a completely closed vesicle. Sections of the lens at this stage show that the cells constituting part of its wall which lies toward the center of the optic cup become elongated to form the lens fibers, (Refer Figure 8.2).

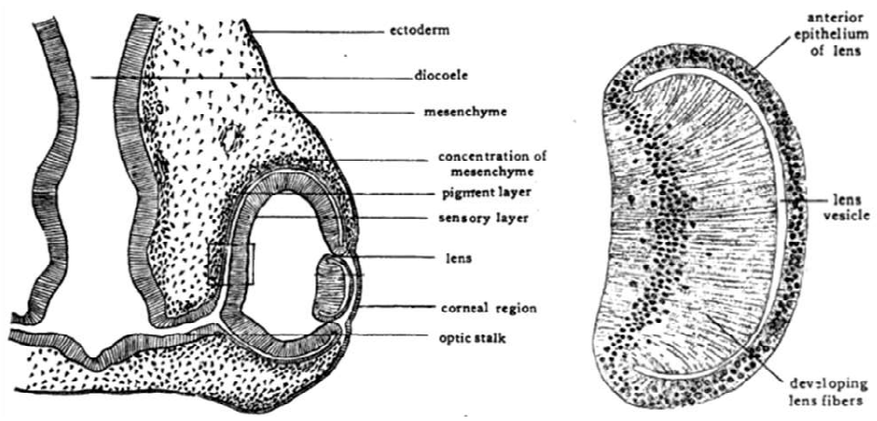

Fig. 8.2 *Structure of the Eye and Lens in a Four-Day Chick*

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At this stage we can identify the beginning of most of the structures of the adult eye. The thickened internal layer of the optic cup gives rise to the sensory layer of the retina. Fibers arise from nerve cells in the retina and grow along the groove in the ventral surface of the optic stalk toward the brain to form the optic nerve. The external layer of the optic cup gives rise to the pigment layer of the retina. Mesenchyme cells seem to aggregate outside the optic cup, from which the sclera and choroid coat are derived. Some of the mesenchyme makes its way into the optic cup through the choroid fissure and gives rise to the cellular elements of the vitreous body. The complex ciliary apparatus of the adult eye is derived from the margins of the optic cup adjacent to the lens. The corneal and conjunctival epithelium arises from the superficial ectoderm overlying the eye. Mesenchyme cells which make their way between the lens and the corneal epithelium give rise to the substantia propria of the cornea.

8.3 DEVELOPMENT OF BRAIN IN CHICK

Formation of the central nervous system appears in chicks of 16 to 18 hours as a local thickening of the ectoderm which forms the neural plate. The neural plate then becomes longitudinally folded to form the neural groove. By fusion of the margins of the neural folds, (first in the cephalic region and later caudally) the neural groove is closed to form a tube and at the same time separated from the body ectoderm. The cephalic portion of the neural tube becomes dilated to form the brain and the remainder of the neural tube gives rise to the spinal cord. In its early stages the brain shows a series of enlargements in its ventral and lateral walls, indicative of its fundamental metameric structure. In the establishment of the three vesicle condition of the brain, the lines of demarcation between prosencephalon, mesencephalon and rhombencephalon are formed by the exaggeration of certain inter-neuromeric constrictions and the obliteration of others. The original neuromeric enlargements persist longest in the rhombencephalon. The three-vesicle condition of the brain is transitory. By forty hours, the division of the rhombencephalon into metencephalon and myelencephalon is clearly indicated.The division of the prosencephalon and the establishment of the five-vesicle condition (characteristic of the adult brain) does not take place until somewhat later.

In Chicks of 55 Hours

Appearance of the cranial flexure has resulted in the bending of the brain so that the entire prosencephalon is displaced ventral and then toward the heart. At the same time the head of the embryo has undergone torsion and lies with its left side on the yolk. Although flexion and torsion have thus completely changed the general appearance of the brain as seen in entire embryos, the regions already established in 40hour chicks are still evident. The prosencephalon has, however, become very noticeably enlarged cephalic to the optic vesicles, and a slight constriction in its dorsal wall indicates the beginning of the demarcation of the telencephalic region from the diencephalic region.

The Formation of Telencephalic Vesicles

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By the end of the third day, the antero-lateral walls of the primary forebrain have been evaginated to form a pair of vesicles lying one on either side of the mid-line. These lateral evaginations are known as the telencephalic vesicles. The openings through which their cavities are continuous with the lumen of the median portion of the brain are later known as the foramina of Monro. The telencephahc division of the brain includes not only the two lateral vesicles but also the median portion of the brain; from which they arise. The teloccele has therefore three divisions, a median, broadly confluent posteriorly with the diocoele and two lateral, connecting with the median through the foramina of Monro,(Refer Figure 8.3).

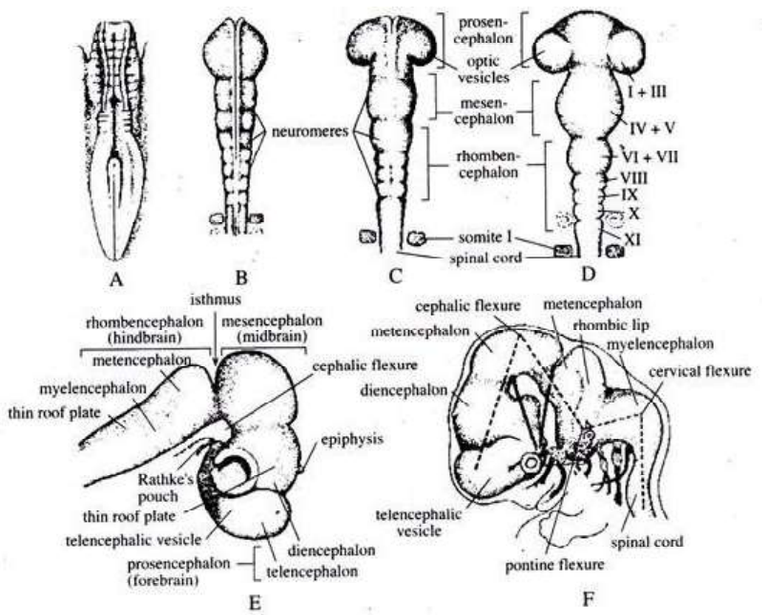

Fig. 8.3 *Early Development of Brain in Chick*

The above Figure 8.3 illustrates the early development of brain in chick: dorsal view with 4 pairs of somites (A); Seven pairs of somites (B); Seven pairs of somites (C); Lateral view of brain at 75 hrs; Lateral view shoeing flexures.

Before the formation of the telencephalic vesicles the most anterior part of the brain lay in the mid-line, but the rapid growth of the telencephalic vesicles soon carries them anteriorly beyond the median portion of the teloccele. The median anterior wall of the teloccele which formerly was the most anterior part of the brain and which remains the most anterior part of the brain lying in the mid-line is known as the lamina terminalis. The telencephalic vesicles become the cerebral hemispheres and their cavities become the paired lateral ventricles of the adult brain. The hemispheres undergo enormous enlargement in their later development and extend dorsally and posteriorly as well as anteriorly, eventually covering the entire diencephalon and mesencephalon under their posterior lobes.

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As a matter of convenience in dealing with the morphology of the brain, more or less arbitrary lines of division between the adjacent brain regions are recognized. The division between telencephalon and diencephalon is an imaginary line drawn from the velum transversum to the recessus opticus, Velum trans versum is the name given to the internal ridge formed by the deepening of the dorsal constriction which was first noted in chicks of 55 hours as indicating the impending division of the primary fore-brain. The recessus opticus is a transverse furrow in the floor of the brain which in the embryo leads on either side into the lumina of the optic stalks, (Refer Figure 8.4).

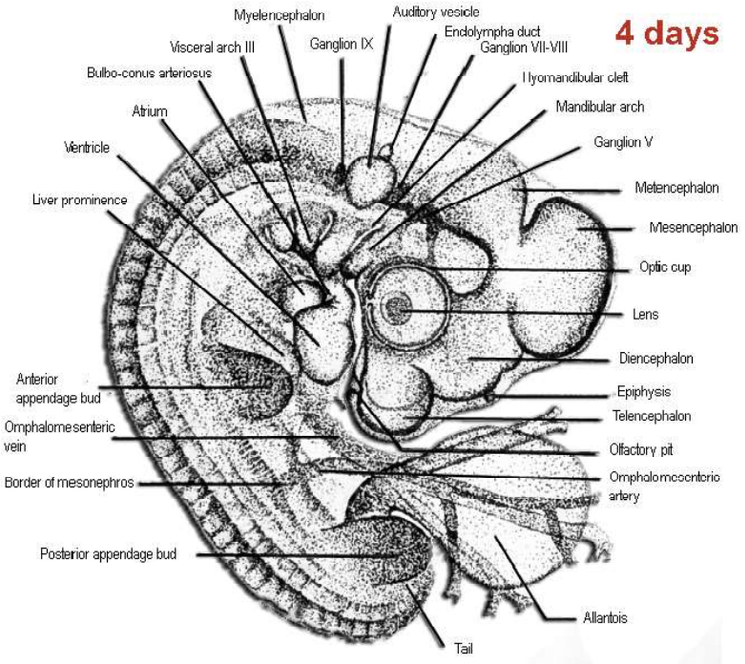

Fig. 8.4 *Median Longitudinal Section of Four-Day Chick*

Diencephalon

The 'lateral walls of the diencephalon at this stage show little differentiation except ventrally where the optic stalks merge into the walls of the brain. Except for some elongation it does not differ from its condition when first formed in embryos of about 55hours. The infundibular depression in the floor of the diencephalon has become appreciably deepened and lies in close proximity to Rathke's pocket with which it is destined to fuse in the formation of the hypophysis. Later in development the lateral walls of the diencephalon become greatly thickened to form the thalami, thus reducing the size and changing the shape of the diocoele, which is known in adult anatomy as the third brain ventricle. The anterior part of the roof of the diencephalon remains thin and by the ingrowths of blood vessels from above is pushed into the third ventricle to form the anterior choroid plexus.

Due to a slight bend in the embryo the section is para-sagittal in the mid-dorsal region but for the most part it passes through the embryo in the sagittal

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plane. The boundary between the diencephalon and the mesencephalon is an imaginary line drawn from the internal ridge formed by the original dorsal constriction between the primary fore-brain and mid-brain, to the tuberculum posterius. The tuberculum posterius is a rounded elevation in the floor of the brain of importance chiefly because it is regarded as marking the boundary between diencephalon and mesencephalon.

Mesencephalon

The mesencephalon as yet shows no specializations, beyond a thickening of its walls. The dorsal and lateral walls of the mesencephalon later increase rapidly in thickness and become the optic lobes (copora quadrigemina) of the adult brain. The optic lobes should not be confused with the optic vesicles arising from the diencephalon of the embryo. They are entirely different structures. The floor of the mesencephalon also becomes greatly thickened and is known in the adult as the crura cerebri. It serves as the main pathway of the fiber tracts which connect the cerebral hemispheres with the posterior part of the brain and the spinal cord. The originally capacious mesocoele is thus reduced by the thickening of the walls about it to a narrow canal (Aqueduct of Sylvius).

Metencephalon

The boundary between the mesencephalon and metencephalon is indicated by the original interneuromeric constriction which separates them at the time of their establishment. The caudal boundary of the metencephalon is not definitely defined. It is regarded as being located approximately at the point where the brain roof changes from the thickened condition (characteristic of the metencephalon) to the thin condition (characteristic of the myelencephalon). The metencephalon shows practically no differentiation in four-day chicks. Later in development there is ventrally and laterally an extensive ingrowth of fiber tracts giving rise to the pons and to the cerebellar peduncles of the adult metencephalon. The roof of the metencephalon undergoes extensive enlargement and becomes the cerebellum of the adult brain.

Myelencephalon

In the myelencephalon the dorsal wall becomes greatly reduced in thickness, indicative of its final fate. Like the roof of the diencephalon, the roof of the myelencephalon later receives a rich supply of small blood vessels by which it is pushed into the myelocoele to form the posterior choroid plexus (choroid plexus of the fourth ventricle). The ventral and lateral walls of the myelencephalon become the floor and side-walls of the medulla of the adult brain.

Ganglia of Cranial Nerves

In the brain region, cells derived from the cephalic portion of the neural crest have become aggregated to form ganglia. The largest and the most clearly defined ganglia present in four-day chicks is the Gasserian ganglion of the fifth (trigeminal) cranial

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nerve. From its cells sensory nerve fibers grow mesiad into the brain and distad to the face and mouth region. In four-day chicks the beginning of the ophthalmic division of the fifth nerve extends from the ganglion toward the eye and the beginning of the mandibulo axillary division is growing toward the angle of the mouth. Immediately cephalic to the auditory vesicle is a mass of neural crest cells which is the primordium of the ganglia of the seventh and eighth nerves. The separation of this double primordium to form the geniculate ganglion of the seventh nerve and the acoustic ganglion of the eighth nerve begins during the fourth day. Posterior to the auditory vesicle the ganglion of the ninth nerve can be clearly seen even in whole-mounts. The ganglia of the tenth (vagus) nerves can be recognized in sections of chicks at the end of the fourth day but are difficult to make out in whole-mounts, (Refer Figure 8.5).

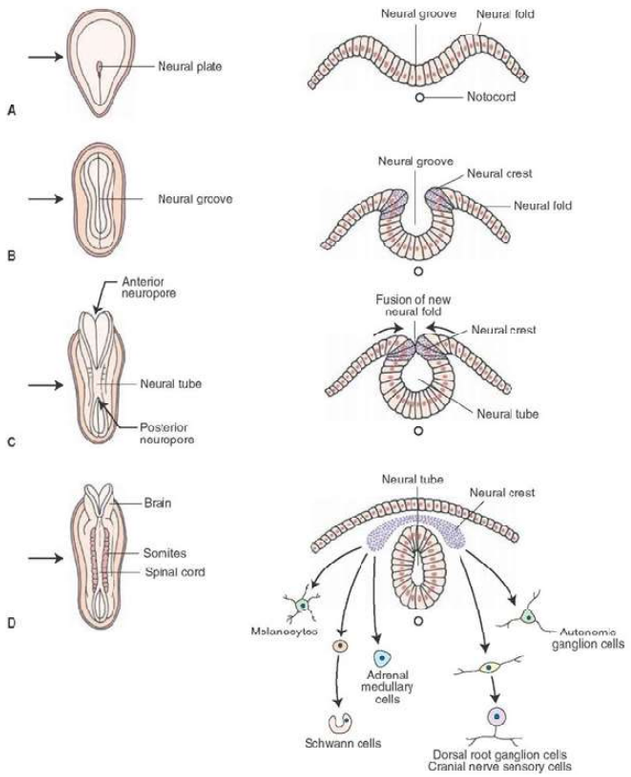

Fig. 8.5 *Origin and Development of Neural Crest, Neural Tube, and Ganglion Cells*

Check Your Progress

1. With what does the vertebrate eye development begin?
2. Where is the neural tube formed from the neural ectoderm located?
3. What does the major development of the eye involve?
4. What leads to the formation of lens?

8.4 DEVELOPMENT OF HEART IN CHICK

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The heart in adult vertebrates is a ventral unpaired structure. Its origin in the chick from paired primordia is correlated with the way the young embryo lie spreaded out on the yolk surface. When the ventral body wall is completed by the folding together of layers which formerly extended to right and left over the yolk, the paired primordia of the heart are brought together in the mid-line. Their fusion establishes the heart as an unpaired structure lying in the characteristic ventral position.

After the fusion of its paired primordial, the heart is a nearly straight, double-walled tube. The primordial endocardium of the heart has the same structure and arises in the same manner as the endothelial walls of the primitive embryonic blood vessels with which it is directly continuous. The epimyocardial layer of the heart is an outer investment which surrounds and reinforces the endocardial wall. As development progresses, the epimyocardium becomes greatly thickened and is finally differentiated into two layers, a heavy muscular layer, the myocardium and a thin non-muscular covering layer, the epicardium, (Refer Figure 8.6).

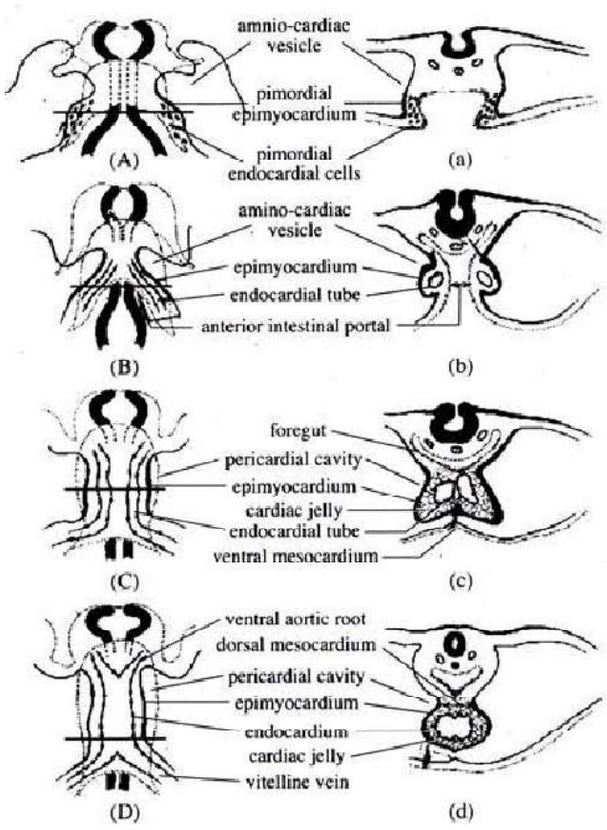

Fig. 8.6 *Chick Embryos showing the Origin and Subsequent Fusion of Paired Primordial of Heart*

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In the apposition of paired primordia of the heart to each other, the splanchnic mesoderm from either side of the body comes together dorsal and ventral to the heart. The double layered supporting membranes thus formed are known as the dorsal mesocardium and the ventral mesocardium respectively. The ventral mesocardium disappears shortly after its formation, leaving the heart suspended in the body cavity by the dorsal mesocardium. Somewhat later the dorsal mesocardium also disappears except at the caudal end of the heart. Thus the heart comes to lie in the pericardial cavity unattached except at its two ends. The cephalic end of the heart remains fixed with reference to the body of the embryo where the ventral aorta lies embedded ventral to the floor of the pharynx and the caudal end of the heart is fixed by the persistent portion of the dorsal mesocardium and the omphalomesenteric veins.

The straight tubular condition of the heart persists but at short time. The unattached ventricular region becomes dilated and is bent out of the mid-line toward the embryo's right while the fixed bulboconus arteriosus and the sinus venosus are held in their original median position. This bending of the heart to form a U-shaped tube begins to be apparent in embryos of 30 hours and becomes rapidly more conspicuous, until by forty hours the ventricular region of the heart lies well to the right of the embryo's body.

The bending of the heart to the side involves a considerable factor of 'mechanical expediency'. The initiation of the bending process depends on the fact that the heart is becoming elongated more rapidly than is the chamber in which it lies fixed by its two ends. The fact that the bending takes place to the side rather than dorsally or ventrally may be attributed to the impediment offered to its dorsal bending by the body of the embryo and to its ventral bending by the yolk.

The lateral bending of the heart attains its greatest extent at about 40 hours of incubation. At this stage, torsion of the body of the embryo changes the mechanical limitations in the heart region. As the embryo comes to lie on its left side, the heart is no longer pressed against the yolk. As a result the bend begins to swing somewhat ventral and lies less closely against the body of the embryo.

At about this stage of development, a new factor affects the changes in the shape of the heart. The closed part of the U-shaped bend is forced caudal and at the same time becomes twisted on itself to form a loop. In the formation of the loop the atrial region is forced slightly to the left (i.e., towards the yolk) and the conus is thrown across the atrial region by being bent to the right (i.e., away from the yolk) and then caudal. The ventricular region constitutes the closed end of the loop. This twisting process reverses the original cephalocaudal relations of the atrial and ventricular regions. The atrial region which was at first caudal to the ventricle now lies cephalic to it as in the adult heart.

The atrial region and the ventricular region which formerly were continuous without any line of demarcation, are by this time beginning to be marked off from each other by a constriction. As both the atrium and the ventricle become enlarged,

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this constriction is accentuated. The constricted region is now termed as the atrioventricular canal.

During the fourth day, the bulboconus arteriosus becomes closely applied to the ventral surface of the atrium. As the atrium grows, it tends to expand on either side of the depression made in it by the pressure of the bulboconus. These lateral expansions of the atrium are the first indication of the division of the atrium into right and left chambers which are later completely separated from each other. At the same time a slight longitudinal groove appears in the surface of the ventricle which indicates the beginning of the separation of the ventricle into right and left chambers. The division of the bulboconus to form the root of the aorta and the pulmonary artery does not appear until a later stage of development.

During the changes in the external shape of the heart which have been described, the whole heart has come to occupy a more caudal position with reference to other structures in the embryo. When the heart is first formed it lies at the level of the rhombencephalon. As development progresses it moves farther and farther caudad until at the end of the fourth day it lies at the level of the anterior appendage buds. Being unattached to the body, the ventricular region of the heart is carried farthest caudad, (Refer Figure 8.8).

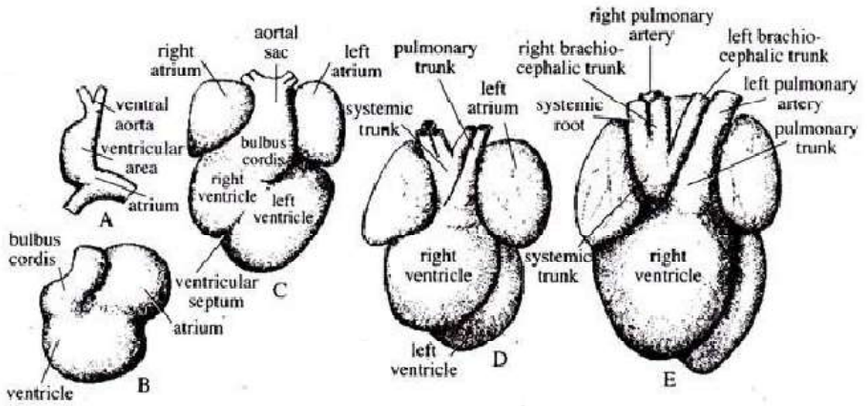

Fig. 8.7 *Development of Heart in Chick (Ventral View): 33-48 Hrs (A); 72 Hrs (B); 4th Day (C); 7th Day (D); 9th Day (E).*

The outer of the two layers shown is the epi-myocardium; the inner, endocardium. The torsion of the embryo's body is going on at the level of the heart. Since torsion involves the more cephalic regions first and progresses caudad the transverse axis of the body of the embryo is at different inclinations to the yolk at the cephalic end and at the caudal end of the heart.

The changes which take place in the heart wall can be seen best in sections. The endocardium in the heart of a four-day chick is still a single cell layer having the lumen. The original epimyocardium at this stage can be differentiated into an inner myocardial portion and an outer epicardial portion. The myocardium has become greatly thickened and the cells in it are elongated and beginning to show

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the histological characteristics of developing muscle cells. Their arrangement in bundles which project toward the lumen fore-shadows the formation of the muscle bands (trabeculae carneae) which ridge the inner wall of the adult heart. The cells of the epicardial portion of the epi-myocardium become flattened to form the epithelial and connective tissue covering of the heart (epicardium). Lying between the endocardium and the myocardium in the region of the atrioventricular canal and of the opening of the ventricle into the bulboconus, there are loosely aggregated cells which are mesenchymal in characteristics. These cells constitute what is called endocardial cushion tissue. They later take part in the formation of the septa which divide the heart into chambers and in the formation of the connective tissue framework of the cardiac valves.

8.5 EMBRYONIC INDUCTION

During the embryonic process, one group of cells (inducing tissue) directs the development of another group of cells (the responding tissue) and the process is known as embryonic induction. In most animal embryos it directs the development of various tissues and organs; for example, eye lens and the heart. During the process of embryonic development in amphibians, dorsal ectodermal cells in mid-longitudinal region get differentiated to form the neural plate, only when chordamesoderm is below it. Chordamesoderm is a layer formed by the invagination cells from the region of dorsal blastoporal lip that forms the roof of the archenteron. The dorsal blastoporal lip of the blastula has the capability to induce the formation of neural plate in ectoderm of the host and the phenomenon is called neural induction. Similarly, other parts of an embryo can induce the formation of many other structures and the process is called as embryonic induction. According to German embryologist, Hans Spemann and his student, Hilde Mangold, dorsal lip of the blastopore is the primary organizer because it is the first in the sequence of inductions and has the capacity to organize development of the second embryo, (Refer Figure 8.8).

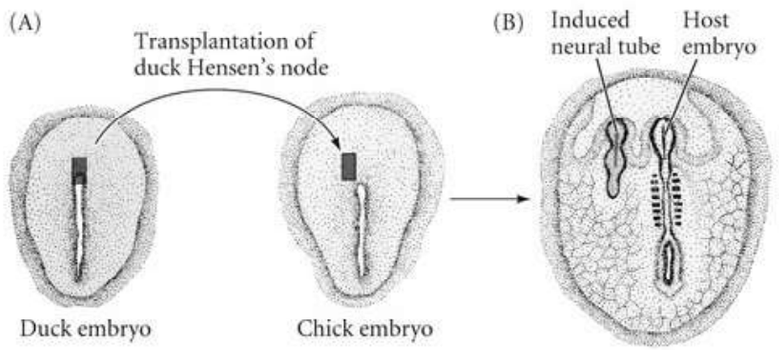

Fig. 8.8 *Embryonic Induction in Chick showing Neural Induction*

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Types of Embryonic Induction

According to Lovtrup, embryonic induction is classified into two basic categories, i.e., endogenous and exogenous inductions.

- **Endogenous Induction:** In this case, some embryonic cells gradually attain new diversification patterns through the inductors produced by them to undergo either self-transformation or self-differentiation. E.g., Mesenchymal cells of ventral pole of Echinoid and in yolk-laden cells of dorsal lip of amphibian blastopore.
- **Exogenous Induction:** In this case, some external agent or a cell or a tissue is introduced into an embryo, which exert their influence by a process of diversification pattern upon neighboring cells through contact induction and the phenomenon is called exogenous induction. It can be homotypic or heterotypic depending on the fact that inductor provokes the formation of same or different kind of tissues respectively. In case of homotypic induction, an inductor is produced by differentiated cell which serves to maintain a proper state of the cell and also crosses the cell boundaries to induce adjacent cells to differentiate according. On the other hand, formation of a secondary embryonic axis by an implanted presumptive notochord in amphibians is a good example of the heterotypic exogenous induction.

Experimental Evidences to Induction

Spemann and Mangold (1924) conducted many experiments to understand the phenomena of embryonic induction.:

- They took heteroplastically a piece of dorsal lip of blastopore of an early gastrula of pigmented newt, *Triturus cristatus.*
- They grafted it near the ventral or lateral lip of blastopore of early gastrula of pigmented newt *T. taeniatus*.
- Most of the graft invaginated into the interior to develop notochord and somites, and thus induced the host ectoderm to form a neural tube, leaving a narrow strip of tissue on the surface.

When the host embryo developed, an additional whole system of organs was induced at the graft placement area. Except for anterior part of head, almost a complete secondary embryo made up of additional organs was formed. Posterior region of the head was present as indicated by a pair of ear rudiments, (Refer Figure 8.9).

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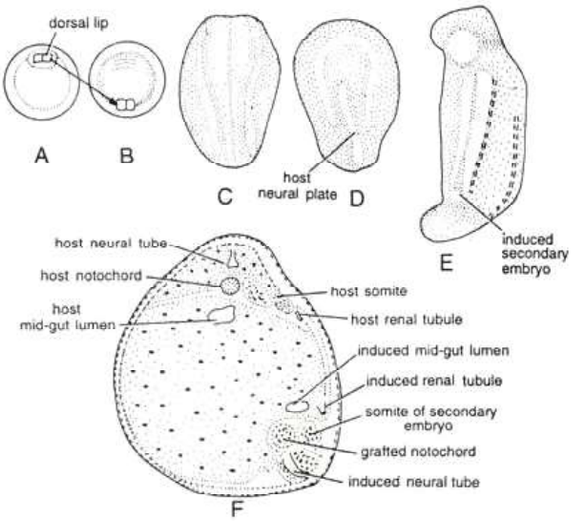

Fig. 8.9 *Induction of Secondary Embryo in Triturus*

The above Figure illustrats the induction of secondary embryo in *Triturus* by transplanting a piece of dorsal lip to future belly region of another gastrula (A,B); stages of resulting embryo (C, D, E); formation of secondary embryo (F).

This experiment involved heteroplastic type of transplantation. So, the notochord of secondary embryo consisted entirely of graft cells and the somites comprises partly of graft and partly of host cells. Some cells, which did not invaginate during gastrulation, remained in neural tube. The bulk of neural tube, part of somites, kidney tubules and ear rudiments of secondary embryo comprises of host cells. The graft became self-differentiated and induced the adjoining host tissue to form spinal cord and other structures including somites and kidney tubules. In 1938, Spemann described dorsal lip of early gastrula as a 'primary organizer' of the process of gastrulation. However, the organization of secondary embryo results due to a combined influence of a series of both inductive interactions and self-differentiative changes in both host and donor tissues. Hence, these days the term 'embryonic induction' or 'inductive interactions' is preferred and the part that is the source of induction is known as 'inductor', (Refer Figure 8.10).

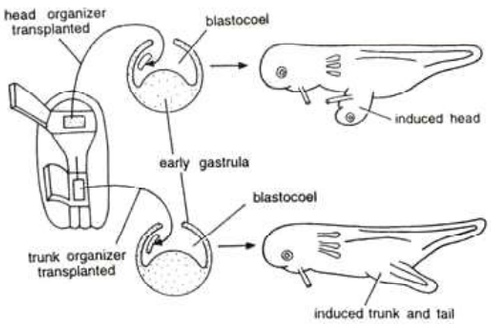

Fig. 8.10 *Separation of Neural Inductor into Head and Trunk*

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Primary Induction and Gray Crescent

At the onset of gastrulation, the dorsal lip region of blastopore can be traced back to the gray-crescent of the undivided fertilized amphibian egg. Some developmental biologists have established that the crescent material of egg cortex can initiated gastrulation and possess the capacity of neural induction. According to Curtis, a change occurs in cortical organization which spreads across the surface of egg during the 2nd and 3rd cleavages; originating from the gray crescent. When this change is accomplished, interactions (probably of a biophysical nature) occur among various parts of the cortex, (Refer Figure 8.11).

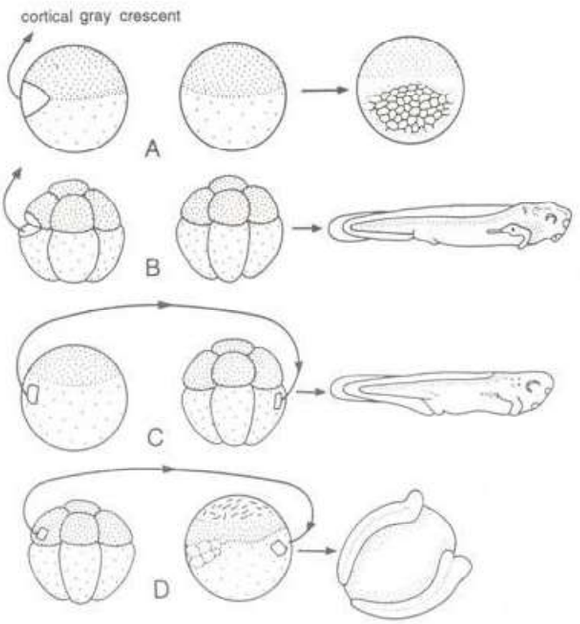

Fig. 8.11 *Experiment of Curtis on* Xenopus

The above Figure shows the experiment of curtis on Xenopus: Excision of gray crescent at one cell stage, no gastrulation (A); Excision of gray crescent at eight cell stage, normal gastrulation (B); Graft of gray crescent one cell stage transplanted to ventral part of eight cell stage, no induction (C); Graft of gray crescent eight cell stage transplanted to ventral margin of one cell stage, induced induction (D).

Mechanism of Neural Induction

Development of the ectoderm overlying the roof of the archenteron into neural tissue suggests a direct action upon the ectodermal cells, either by surface interaction or by chemical mediation. One of the broad possibilities is surface interaction of the cells at the inductive interface. Another broad possibility is a chemical mediation of the inductive effect. Studies have found that few inorganic agents such as kaolin and iodine, local injury, exposure to saline solutions of excessively low or high pH

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leads to neural differentiation in ectoderm. Neural induction occurs at the time when the material of chordamesoderm moves from the dorsal lip of blastopore inward and forward (Saxen and Toivonen 1962). The inductive stimuli exhibit a time gradient, which may be crucial with regard to action and reaction events. There are following theories related to mechanism of neural induction:

- **Protein Denaturation Theory of Neural Induction:** In 1963 Ranzi, found that neural induction and notochord formation are related to protein denaturation. In amphibian, gray crescent is the site of notochord formation which is a center of high metabolic activity and corresponds to sites of protein denaturation.
- **Gradient Theory of Neural Induction:** Toivonen (1968) and Yamada (1961) affirmed that 2 chemically distinct factors are concerned in the action of primary inductor. Out of these 2 factors, one is neuralizing agent and another is mesodermalizing agent, (Refer Figure 8.12).

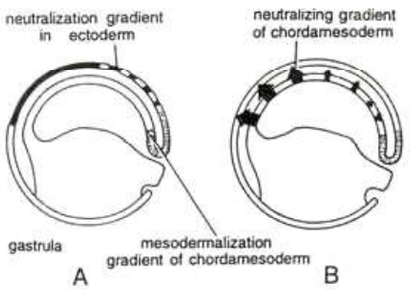

Fig. 8.12 *Distribution of Neutralization and Mesodermalization Gradient*

- **One Factor Hypothesis of Neural Induction:** It was given by Nieuwkoop in 1966, using living notochord as an inductor. It states that there is only one factor which first evokes ectoderm to form neural tissue and later causes ectoderm to transform into more posterior and mesodermal structure during neural induction, (Refer Figure 8.13).

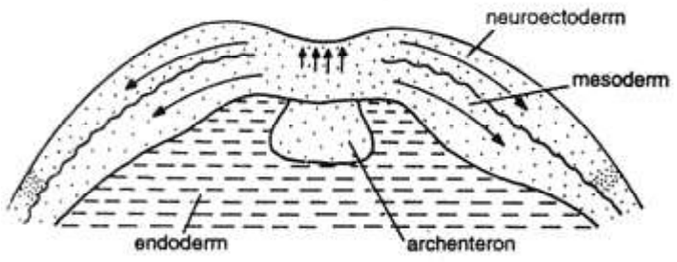

Fig. 8.13 *Medio-Lateral Speading of an Inductive Action within the Mesoderm and a Similar Spreading of Neutralizing Action in Overlying Ectoderm*

- **Ionic Theory of Neural Induction:** Barth and Barth (1969) stated that the actual process of induction may be initiated by the release of ions from bound form, representing an alteration in the ratio between bound to free

ions within the cell of early gastrula. Induction of nerve and pigment cells in small aggregates of prospective epidermis of the frog gastrula is dependent on the concentration of sodium ions. Normal embryonic induction depends on endogenous source of ions, i.e., intracellular release of ions that occurs during late gastrulation.

- **Genic Basis of Neural Induction:** Studies have shown that the component tissues of neural inductor become differentiated prior to ectodermal cells. During this process, the rate of transcription of mRNA and differential activation of genes becomes many folds, while the differentiation of ectodermal cells is set in only after mid-gastrulation. This advocates genetic basis of neural induction.

Embryonic Induction in Different Chordates

The neural induction was first discovered in urodele amphibians, but it has been established that the dorsal lip of blastopore and the roof of the archenteron of other vertebrates shows same function. Chordamesoderm in all the vertebrates induces the nervous system and sense organs. In Cyclostomes, especially in lampreys, the property of neural induction lies in the presumptive chordamesodermal cells of dorsal lip of blastopore. In *Amphioxus* the chordal tissue of gastrula has the power of neural induction, while mesodermal and endodermal tissues have little such inductive power,(Refer Figure 8.14).

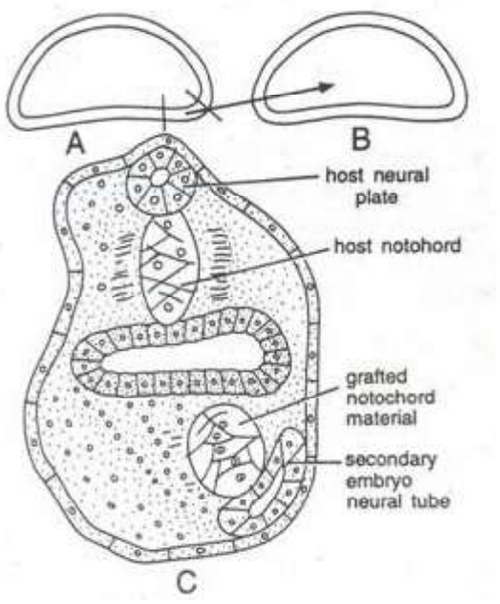

Fig. 8.14 *Neural Induction in* Amphioxus

In bony fishes, the induction of secondary well developed embryos was produced by transplanting the posterior edge of the blastodisc which corresponds to the dorsal lip of the blastopore, into the blastocoel of another embryo or by

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transplanting the chordamesoderm and ectoderm. Transplanting the dorsal lip of the blastopore in the sturgeon also produced neural inductions. In frogs, the induction of secondary embryo can be formed by the dorsal lip of the blastopore transplanted into the blastocoel of a young gastrula, as in case of newts and salamanders. In reptiles archenteron has the same inducing activity as in other vertebrates but there is no experimental proof of the occurrence of neural inductor, (Refer Figure 8.15).

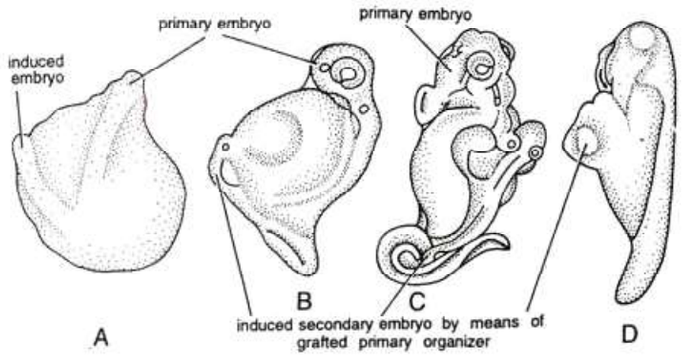

Fig. 8.15 *Induction of Secondary Embryos by means of Grafted Primary Organizers in Lampray (A); Pearch (B, C) and Frog (D)*

In birds the existence of primary organizer was established by Waddington and co-workers. Anterior half of the primitive streak was the inducing part similar to the lips of the blastopore in amphibians. A neural induction was performed successfully in a rabbit embryo by cultivating the early blastodisc on a plasma clot and implanting the primitive streak of the chick as inductor. Tissues of the mammalian gastrula were found having competence for neural induction. Moreover, anterior end of a rabbit embryo, with two pairs of somites, induced a neural plate in a chick embryo when placed under a chick blastoderm.

Other types of Embryonic Induction

Besides gastrulation growth, various organ systems of the embryo begin to differentiate and acquire the power of inducing the differentiation of structures or organs such as eyes, ears, limbs and lungs, etc. These organs develop organizing property and become the source of induction. This series of organizers is known as secondary, tertiary and quaternary organizers. Progressive development of embryonic organs depends upon sequential induction. One embryonic tissue interacts with the adjacent tissues; induces it to develop and the process continues in sequence. In anterior part of embryo, chordamesoderm which is the primary organizer induces the formation of fore-brain and optic area. The optic area evaginates to form the optic vesicle. By invagination it changes into a double walled cup-like structure, the optic cup which acts as secondary organizer to induce the

formation of tertiary organizer for the formation of cornea. The layer of mesenchyme left in front of anterior chamber of eye combines with the overlying somatic ectoderm (epidermis) and forms the cornea, choroid and sclera. Thus the whole process of development occurs due to induction and interaction only. Numbers of inductions are secondary or tertiary such as nasal-groove, optic vesicle, lens, cornea and so on involve ectodermal reactions.

Check Your Progress

5. What is known as induction action or organizer action?
6. What structure is the heart in adult vertebrates?
7. At how many hours of incubation does the lateral bending of the heart attains its greatest extent?
8. Where can one see the changes which take place in the heart wall?

8.6 ORGANIZER CONCEPT IN EMBRYOLOGY

The effect of embryonic interaction or organizer is a morphogenetic effect by which one organic tissue transmits a chemical substance that influences other embryonic part to produce a structure that otherwise could not come into existence. The embryonic tissue which exerts such an influence is called an inductor and the chemical substance secreted by an inductor is known as evocators. The tissue on which evocator works and the tissue responses is known as responsive tissue. The action of the indicator through evocator is known as induction action or organizer action. This process of induction influences greatly the protein synthesis mechanism of responsive tissues as a result of which definite structure forming cells become very active.

Characteristics of the Organizer

It is capable of self-differentiation and organization. It has the power to induce the changes within the cell and to organize surrounding cells, together with the induction and early organization of neural tube. Primary organizer determines the main features of axiation as well as the organization of vertebrate embryo. The early gastrula (dorsal blastopore lip) contains the archenteric and deuterocephalic produce head organs, while late gastrula (dorsal blastopore lip) contains the spinocaudal organizer produce trunk and tail organs.

Origin of the Concept of Organizer

A German embryologist Hans Spemann and his student Hilde Mangold performed transplantation experiment on a newt *Triturus cristatus*, an Urodela of class Amphibia. Spemann grafted a piece taken from the dorsal lip of early gastrula

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of *Rana* sp. to the lateral lip region of the early gastrula of *Triturus cristatus*. The embryo of *Rana* sp. is donor and the embryo of *Triturus* is the host. They observed that the cells of the grafted piece enter into the gastrula and form notochord and somites. In this embryo its own dorsal lip of blastopore forms neural groove, notochord, mesoderm etc. Similarly the grafted tissue influences to form notochord, neural groove and mesoderm. That is in the same embryo double set of notochord, nerve cord and mesoderm are produced. In this case donor tissue has secreted some chemical substances which has induced to form neural groove, notochord etc. in the host embryo. The donor tissue had pigments and the induced neural groove has also colored pigments. After the completion of the gastrulation they observed that a larva has developed with two heads. One head is due to normal development and the other head production has been induced by donor tissue. They examined the larva under the microscope and found that notochord, renal tubules, gut etc. have been formed by the tissue of the host embryo as a secondary set. If the donor tissue would not have been grafted such secondary structures wound not develop. From this experiment they concluded that dorsal lip of the donor had influenced greatly the tissue and thus has brought about change in the host tissue development. If it is not the fact then how a head had developed in the abdomen of the host? This secondary head formation is due to induction effect of donor tissue. This process of influencing other tissue was termed as induction by Spemann and the tissue that induced the tissue was known as the inductor or organizer.

Primary Organizer

Spemann continued his grafting experiments taking tissues from different zones of the gastrula and observed that except dorsal lip of the early gastrula other zone of tissue cannot create any induction effect but when dorsal lip is grafted a complete embryo is formed. He named the dorsal lip as organizer as this dorsal lip organizes the developmental process of the embryo. According to him this dorsal lip induces to form neural tube and the neural tube then induces to form the eyes. The dorsal lip is composed of chorda-mesoderm and as it primarily acts as inducer so he named the dorsal lip or chordamesoderm as primary organizers.

Secondary, Tertiary and Quaternary Organizers

As the gastrulation proceeds due to primary organizer's induction primary organs begin to form and the early stages of organ development are known as organ rudiments. These organ rudiments themselves may act as organizer and then they are known as secondary organizer. Tissues formed by the action of secondary organizer may in turn induce further development. Then they are known as tertiary organizer. These successive stages of organizer activities start from the primary organizer.

How these organizers act in succession can clearly be understood from the examples of the development of eye in amphibian, chick etc. First of all due to

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induction effect of the primary organizer forebrain and within the forebrain eye forming cells are produced. These cells push out as a vesicle outside the forebrain. These vesicles are known as optic vesicle. This vesicle grows through the lateral mesenchyme and reaches the epidermis. As soon as the vesicle comes in contact with the epidermis the outer layer of the vesicle invaginates to form a double layered optic cup. The inner layer of the optic cup is formed of sensory cells and the outer layer is formed of pigmented cells. They two together form the retina. The chemical substances secreted by the optic cup induce to form the lens between the optic cup and the epidermis. The peculiar thing is that if the optic vesicle is prevented from coming in contact with the epidermis there will be no lens formation. So the optic cup acts as secondary organizer. Similarly lens and retina together induce to form cornea so lens and retina together act as tertiary organizer and so on. On the basis of various experimental evidences Lehmon (1945) said that specific regionality of induction effects present in the dorsal lip of the blastopore. He further said that the roof of the archenteron definitely possess specific induction activities for the differentiation of head and trunk regions. On the basis of the regional specificity he classified the inductors into three groups. They are:

- **Archenocephalic Inductor,** due to induction effect of this inductor partial head, fore-brain, eye, nasal cavities are formed.
- **Deuterencephalic Inductor,** by its induction effect posterior portion of the head, ear cavities etc. are formed (as arechenocephalic and deuterencephalic inductors induce the formation of different parts in the head region so they together are known as cephalic or head inductors).
- **Spino-Caudal Inductor, t**heir inductive influence leads to the formation of spinal cord and different structures of the tail region.

Check Your Progress

9. What gives rise to the sensory layer of retina?
10. Which cells in amphibians get differentiated to form the neural plate?

8.7 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Vertebrate eye development begins with the formation of the optic vesicles as outgrowths of the forebrain.
2. The neural tube formed from the neural ectoderm is located dorsally in the median plain of the embryo and forms the basis for the central nervous system.
3. The major development of the eye involves ectoderm, neural crest cells, and mesenchyme.

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4. Lens formation may result from internal contractile forces as well as from forces exerted by surrounding cells.
5. The action of the indicator through evocator is known as induction action or organizer action.
6. The heart in adult vertebrates is a ventral unpaired structure.
7. The lateral bending of the heart attains its greatest extent at about 40 hours of incubation.
8. The changes which take place in the heart wall can be seen best in sections.
9. Internal layer of optic cup gives rise to the sensory layer of retina.
10. Dorsal ectodermal cells in amphibians get differentiated to form the neural plate.

8.8 SUMMARY

- The primary optic vesicles arise in chicks of about 30 hours as dilations in the lateral wall of the prosencephalon.
- Formation of the central nervous system appears in chicks of 16 to 18 hours as a local thickening of the ectoderm which forms the neural plate.
- Vertebrate eye development begins with the formation of the optic vesicles as outgrowths of the forebrain.
- The primordia of the lens and neural retina become adherent, as a result of changes in the extracellular matrix, before invagination commences.
- Lens formation may result from internal contractile forces as well as from forces exerted by surrounding cells.
- The neural tube formed from the neural ectoderm is located dorsally in the median plain of the embryo and forms the basis for the central nervous system.
- The prosencephalon is induced by prechordal plate mesoderm and the deuteroencephalon by the anterior portion of the notochordal mesoderm, both lying beneath the primordial brain.
- The heart is among the earliest, largest, and most easily studied organs to form during embryogenesis.
- Embryonic induction describes the embryonic process in which one group of cells, the inducing tissue, directs the development of another group of cells, the responding tissue.
- The embryonic tissue which exerts such an influence is called an inductor and the chemical substance secreted by an inductor is known as evocators.
- The action of the indicator through evocator is known as induction action or organizer action.

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- The primary optic vesicles arise in chicks of about 30 hours as dilations in the lateral wall of the prosencephalon.
- In chicks of four days the choroid fissure has become narrowed by the growth of the walls of the optic cup on either side of it.
- The thickened internal layer of the optic cup gives rise to the sensory layer of the retina.
- Fibers arise from nerve cells in the retina and grow along the groove in the ventral surface of the optic stalk toward the brain to form the optic nerve.
- The 'lateral walls of the diencephalon at this stage show little differentiation except ventrally where the optic stalks merge into the walls of the brain.
- The mesencephalon as yet shows no specializations, beyond a thickening of its walls. The dorsal and lateral walls of the mesencephalon later increase rapidly in thickness and become the optic lobes (copora quadrigemina) of the adult brain.
- The 'lateral walls of the diencephalon at this stage show little differentiation except ventrally where the optic stalks merge into the walls of the brain.
- The mesencephalon as yet shows no specializations, beyond a thickening of its walls. The dorsal and lateral walls of the mesencephalon later increase rapidly in thickness and become the optic lobes (copora quadrigemina) of the adult brain.
- The straight tubular condition of the heart persists but at short time. The unattached ventricular region becomes dilated and is bent out of the mid-line toward the embryo's right while the fixed bulboconus arteriosus and the sinus venosus are held in their original median position.
- The ventral mesocardium disappears shortly after its formation, leaving the heart suspended in the body cavity by the dorsal mesocardium.
- The bending of the heart to the side involves a considerable factor of 'mechanical expediency'.
- The lateral bending of the heart attains its greatest extent at about 40 hours of incubation.
- The atrial region and the ventricular region which formerly were continuous without any line of demarcation, are by this time beginning to be marked off from each other by a constriction.
- Most of the graft invaginated into the interior to develop notochord and somites, and thus induced the host ectoderm to form a neural tube, leaving a narrow strip of tissue on the surface.
- Development of the ectoderm overlying the roof of the archenteron into neural tissue suggests a direct action upon the ectodermal cells, either by surface interaction or by chemical mediation.

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- As the gastrulation proceeds due to primary organizer's induction primary organs begin to form and the early stages of organ development are known as organ rudiments.
- The inner layer of the optic cup is formed of sensory cells and the outer layer is formed of pigmented cells. They two together form the retina.
- By fusion of the margins of the neural folds, (first in the cephalic region and later caudally) the neural groove is closed to form a tube and at the same time separated from the body ectoderm.
- The heart in adult vertebrates is a ventral unpaired structure. Its origin in the chick from paired primordia is correlated with the way the young embryo lies spreaded out on the yolk surface. After the fusion of its paired primordial, the heart is a nearly straight, double- walled tube.
- The neural induction was first discovered in urodele amphibians, but it has been established that the dorsal lip of blastopore and the roof of the archenteron of other vertebrates shows same function.
- The effect of embryonic interaction or organizer is a morphogenetic effect by which one organic tissue transmits a chemical substance that influences other embryonic part to produce a structure that otherwise could not come into existence.

8.9 KEY WORDS

- **Endogenous induction:** It is a certain case, where some embryonic cells gradually attain new diversification patterns through the inductors produced by them to undergo either self-transformation or self-differentiation.
- **Exogenous induction:** In this case, some external agent or a cell or a tissue is introduced into an embryo, which exert their influence by a process of diversification pattern upon neighboring cells through contact induction and the phenomenon is called exogenous induction.
- **One factor hypothesis of neural induction:** It is a hypothesis given by Nieuwkoop in 1966, using living notochord as an inductor. It states that there is only one factor which first evokes ectoderm to form neural tissue and later causes ectoderm to transform into more posterior and mesodermal structure during neural induction
- **Ionic theory of neural induction:** Barth and Barth (1969) stated that the actual process of induction may be initiated by the release of ions from bound form, representing an alteration in the ratio between bound to free ions within the cell of early gastrula.

8.10 SELF-ASSESSMENT QUESTIONS AND EXERCISES

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Short Answer Questions

1. Write a short note on the ionic theory of neural induction.
2. What do you mean by exogenous induction?
3. Discuss the structure of the eye and lens in a four-day chick.
4. Write a note on the development of brain in chick.
5. Draw a diagram of median longitudinal section of four-day chick.
6. Write a note on the ganglia of cranial nerves.

Long Answer Questions

1. Explain mechanism of embryonic induction in different types of gastrula.
2. Explain the role of organizer during development of an embryo.
3. Write a short note on development of diencephalon and mesencephalon.
4. Describe the development of eye in chick.
5. Write a detailed note on embryonic induction.

8.11 FURTHER READINGS

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UNIT 9 FOETAL MEMBRANES, PLACENTA AND GENE THEORY

Structure

9.0 INTRODUCTION

During the development of chick and other vertebrates, certain specialized embryonic tissues or structures are produced that temporarily or permanently do not enter into the formation of the embryo themselves. These are external and devoted in one way or another to the care and maintenance of the developing embryo. These structures are collectively termed as extra-embryonic membranes or foetal membranes or extra-embryonic sacs. These are not precursors of any of the organs of the adult or the larva but serve to assure the requirements of the embryo in connection with nutrition, gas exchange, removal or storage of waste materials and protection.

The extra-embryonic membranes have developed to make the eggs capable of developing on dry land. The eggs of reptiles, birds and prototherian mammals have a protective shell around it. In some reptiles and eutherian mammals the shell has given way to uterine development, but the basic form and function of the extra-embryonic membranes has remained the same. Extra-embryonic membranes are the membranes formed of embryonic tissues, which extend out and beyond the strict confines of the embryonic body and are adapted to fulfill the care and maintenance of the developing embryo. In chick, the presence of an enormous amount of yolk and embryonic life to be spent within a shell is correlated with the development of extra-embryonic membranes. Original blastoderm is a small disc, which spreads by peripheral expansion and eventually covers the entire surface of the egg. But only the most central region is directly connected with the formation of the embryo proper. All the remainder of the blastoderm is extra-embryonic.

In this unit, you will study about the foetal membrane in chick and placenta in mammals, origin of gene theory, nuclear transplantation, differential gene activation, and the various factors involved in teratogenesis in detail.

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9.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand the foetal membranes in chick
- Know the placenta and placentation in mammals
- Learn about nuclear implantation and differential gene activation
- Understand the teratogenesis, and factor involved in teratogenesis

9.2 FOETAL MEMEBRANES IN CHICK

The embryo of chick possesses four extraembryonic or foetal membranes: namely, the yolk sac, the allantois, the amnion and the serosa or chorion. In amphibian embryo, the yolk sac and the allantois are present in rudimentary condition. The amnion and the serosa are developed in the reptilian embryo for the first time in evolutionary history of the vertebrates. In birds these two structures are retained while in mammals these are also present in a modified form. All the extraembryonic membranes are discarded at hatching while the yolk sac is incorporated into the small intestine. As development goes on, the closely set ectoderm and somatopleure (somatic mesoderm) as well as the endoderm and splanchnopleure (splanchnic mesoderm) extend into the extraembryonic area. The developing embryo becomes located at the central area of the blastodisc. These folds initiate with the formation of crescentic head fold which extends backward as the body folds. The body folds subsequently merge with the tail fold. By this, way the embryo undercuts and separates itself from the underlying yolk mass.

In all viviparous animals the embryonic development takes place inside the uterus of the mother, because the eggs are microlecithal and the amount of stored yolk is not sufficient to cope up the needs of the developing embryo. Such embryos get attached to the uterine wall to draw essential substances from the maternal circulation through the placenta. A placenta is an organ built up of maternal and foetal tissues jointly. It serves for the transport of nutrients from the mother tissues with those of the embryo as well as the exchange of gases between the tissues of the two. Thus a placenta may be defined as a temporary connection between the maternal and foetal tissues for the purpose of shelter, nutrition, respiration, excretion and defense.

Mendel called them *bildungsfähigen Elemente,* 'form-building elements'; we call them genes. It is in Mendel's term, however, that we see how closely intertwined were the concepts of inheritance and development in the nineteenth

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century. Mendel's observations, however, did not indicate where these hereditary elements existed in the cell, or how they came to be expressed. The gene theory that was to become the cornerstone of modern genetics originated from a controversy within the field of physiological embryology. In the late 1800s, a group of scientists began to study the mechanisms by which fertilized eggs give rise to adult organisms. Two young American embryologists, Edmund Beecher Wilson and Thomas Hunt Morgan, became part of this group of 'physiological embryologists', and each became a partisan in the controversy over which of the two compartments of the fertilized egg (the nucleus or the cytoplasm) controls inheritance. Morgan allied himself with those embryologists who thought the control of development lay within the cytoplasm, while Wilson allied himself with Theodor Boveri, one of the biologists who felt that the nucleus contained the instructions for development. It is declared that the processes of meiosis, mitosis, fertilization, and unicellular regeneration (only from the fragment containing the nucleus) 'converge to the conclusion that the chromatin is the most essential element in development. He did not shrink from the consequences of this belief. Now, chromatin is known to be closely similar, if not identical with, a substance known as nuclein, which analysis shows to be a tolerably definite chemical composed of a nucleic acid (a complex organic acid rich in phosphorus) and albumin. And thus we reach the remarkable conclusion that inheritance may, perhaps, be affected by the physical transmission of a particular chemical compound from parent to offspring'.

Nuclear transplantation is a method in which the nucleus of a donor cell is relocated to a target cell that has had its nucleus removed (enucleated). Nuclear transplantation has allowed experimental embryologists to manipulate the development of an organism and to study the potential of the nucleus to direct development. Nuclear transplantation, as it was first called, was later referred to as somatic nuclear transfer or cloning. Yves Delage first wrote about nuclear transplantation in 1895, speculating that if one were to replace an egg nucleus with another egg's nucleus, full development would occur. Later in 1938, Hans Spemann suggested an experiment whereby, using technologies not yet available to him, one could remove the nucleus of an egg and replace it with a different nucleus extracted from a developed cell. Thomas King and Robert Briggs were the first to perform experimental nuclear implantation. The technique was soon after used by John Gurdon and eventually led to the first clone of a mammal, 'Dolly' the sheep, by Ian Wilmut in 1996. While biological systems operated from a common genome can be conserved in various ways, they can also manifest highly diverse dynamics and functions. This is because the same set of genes can interact differentially across specific molecular contexts. For example, differential gene interactions give rise to various stages of morphogenesis during cerebellar development. However, after over a decade of efforts toward reverse engineering biological networks from high-throughput omic data, gene networks of most organisms remain sketchy. This hindrance has motivated us to develop comparative modeling to highlight conserved and differential gene interactions across experimental conditions, without reconstructing complete gene networks first.

Teratogenesis is a prenatal toxicity characterized by structural or functional defects in the developing embryo or fetus. It also includes intrauterine growth retardation, death of the embryo or fetus, and transplacental carcinogenesis (in which chemical exposure of the mother initiates cancer development in the embryo or fetus, resulting in cancer in the progeny after birth). Intrauterine human development has three stages: implantation, postimplantation, and fetal development. The first two stages are the embryonic stages and last through the first eight weeks after conception. The fetal stage begins in the ninth week and continues to birth. Depending on the developmental stage, chemical exposure in the mother can result in different degrees of toxicity in the embryo or fetus. In the preimplantation period, a toxic chemical can kill some of the cells in the blastocyst, resulting in the death of the embryo. During the postimplantation period, chemical-induced cell death leads to one of two outcomes. If death is confined to those cells undergoing active cell division at the moment, the corresponding organs are affected, resulting in malformation. If the cell death is generalized without significant replication by the remaining cells to sustain life, the embryo dies. During the third, fetal, period, chemical injury can retard growth or, if severe enough, kill the fetus.

Types of Foetal Membranes in Chick

There are four foetal membranes in chick as described below:

- **Yolk Sac:** It is the most primitive structure containing network of blood vessels and encloses the yolk of the egg. A yolk sac is also present in those fishes which have megalecithal eggs. Despite the lack of stored yolk in mammalian eggs (except in prototherians), the yolk sac has been preserved, as it serves many important secondary functions.
- **Amnion:** The amnion is a thin membrane which eventually encloses the entire developing embryo in a fluid-filled sac. Reptiles, birds and mammals possessing this amnion are often called amniotes, while fishes and amphibians, lacking it, are collectively called anamniotes.
- **Chorion (Serosa):** Chorion is a very thin membrane and it covers the embryo and other extra-embryonic membranes. It is formed by the fusion of the amniotic folds over the embryo. All these extra-embryonic membranes are composite structures as they involve two germ layers.
- **Allantois:** Allantois serves as an excretory and respiratory structure. It is a large sac like structure in reptiles and birds, while its role in mammals varies with the efficiency of the interchange that takes place at the foetal-maternal interface. In pig embryo, the allantois rivals that of the bird's in both size and functional importance, while the allantois in human has been reduced to a mere vestige which contributes only as a well-developed vascular network to the highly efficient placenta.

The amnion and chorion are made up of extra-embryonic ectoderm and somatic layer of mesoderm, while the yolk sac and allantois are composed of extra-embryonic endoderm and splanchnic layer of mesoderm.

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Development of Extra-Embryonic Membranes

During neurulation (neural tube formation) of chick, the lateral plate mesoderm splits into an outer somatic layer of mesoderm lying at the inner side of the ectoderm and an inner splanchnic layer of mesoderm lying outer to the endodermal layer. Both these mesodermal layers enclose a coelomic space between them. The somatic layer of mesoderm and ectoderm are collectively known as somatopleure, while the splanchnic layer of mesoderm along with endoderm forms the splanchnopleure. At the time of development of the avian blastoderm, the somatopleure and splanchnopleure gradually spread peripherally over the yolk mass, far beyond the area where the body of the embryo is taking form. Shortly, the embryo proper begins to be undercut by a series of body folds that serve to delimit the embryonic regions from the more peripheral extra-embryonic somatopleure and splanchnopleure. After the formation of the body folds, the somatopleure and splanchnopleure of chick develop into the four extra-embryonic membranes, (Refer Figure 9.1).

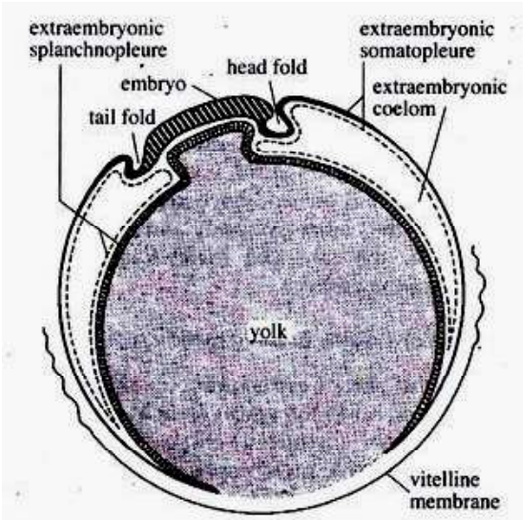

Fig. 9.1 *Schematic Presentation of Extra Embryonic Areas in Chick*

- **Development of Yolk Sac:** The yolk sac is the first extra-embryonic membrane to make its appearance. As the early blastoderm expands, the extra-embryonic splanchnopleure continues to spread over the yolk mass and eventually encloses the yolk completely to form the yolk sac. Coincidentally, the intra-embryonic splanchnopleure is subjected to superficial body folds, which serve to establish a walled digestive tract or gut, in the body of the embryo. The middle of the gut (mid gut) remains connected with the yolk sac by a narrow yolk stalk, where the walls of the gut are continuous with the walls of the yolk sac. Although the yolk sac is connected with the digestive tract by the yolk stalk, the yolky food reserves are not transmitted to the embryo by this route. Rather, the digestion of the

yolk is done by the endodermal lining of the yolk sac through the mediation of appropriate enzymes. In chick, on the 2nd and 3rd day of incubation, networks of blood vessels develop on the inner part of the area opaca, which becomes the area vasculosa. The outer part of area opaca is called the area vitellina.

All the blood vessels of area vasculosa communicate with each other and are joined together on the periphery by the terminal sinus, which incidentally forms the boundary between area vasculosa and area vitellina. The network of the area vasculosa becomes prolonged into the area pellucida and eventually establishes connection with the embryo proper. At about the middle of the 2nd day of incubation, the heart of the embryo begins to beat. Between the 38th and 40th hours of incubation the blood starts circulating through the network of the yolk sac. The blood vessels in the area vasculosa penetrate deep into the yolk. The endodermal surface of the yolk sac is thrown into folds that penetrate the yolk mass.

Then, through the action of appropriate digestive enzymes secreted by the endodermal cells, the yolk is digested or made soluble and is ultimately absorbed by the endodermal lining of the yolk sac. Also during the growth of the allantois, the albumen is forced towards the distal end and gets surrounded by an extension of the yolk sac. It is absorbed along with yolk and transferred by way of the extra-embryonic circulation to the embryo. The entire yolk is not completely absorbed during embryonic life. On the 19th day when the period of incubation is nearing its end, the remains of the yolk sac are enclosed within the body walls of the embryo. During the first 6 days after hatching, the resorption of the remaining part of the yolk sac and yolk gets completed. This remaining yolk reserves are vital to the newly hatched chick while it is adapting to a free-living existence and is developing its feeding behavior, (Refer Figure 9.2).

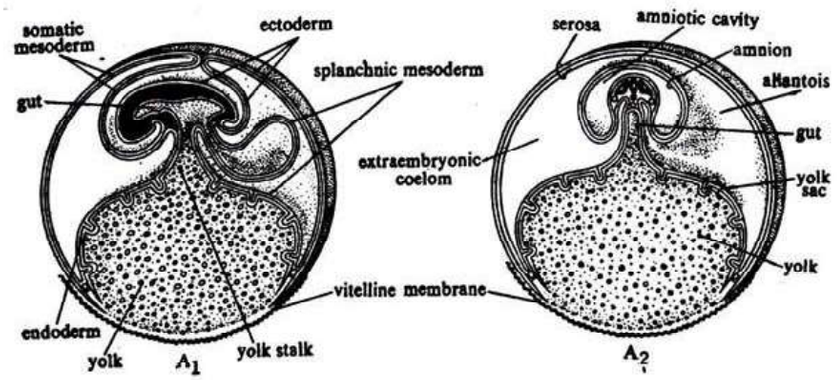

Fig. 9.2 *Formation of Foetal Membrane at an Early Stage of Chick Embryo*

- **Development of Amnion and Chorion:** The amnion and chorion are developed simultaneously and both are derived from the extra-embryonic somatopleure. At about the 30th hour of incubation, the head of the embryo

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sinks somewhat into the yolk and at the same time the extra-embryonic somatopleure is elevated over the embryo by a folding process consisting essentially of a doubling of the somatopleure upon itself. The initial elevation is over the head end of the embryo, producing a double somatopleuric hood, called the cephalic amniotic fold or head fold of the amnion. From a dorsal aspect, the margin of this fold is crescentic in shape, with its concavity directed towards the head of the embryo. As the embryo increases in length, its head grows forward into the amniotic fold.

As the cephalic amniotic fold gradually extends backward, towards the tail region, its caudally extending side limbs called as lateral amniotic folds arch over the embryo from each side to be joined finally by a similar fold or elevation from the tail region called the caudal amniotic fold or tail fold. All these amniotic folds finally converge at the midline, so as to encase the embryo by two sheets of somatopleure from all the sides except from the region of the yolk stalk. The place where all the amniotic folds meet is called the seroamniotic connection or the amniotic raphe, which is a scar like thickening. The seroamniotic connection opens before the hatching of the embryo and admits albumen into the amniotic cavity.

The fusion of the amniotic folds results in the formation of two sac-like membranes and two cavities. The inner somatopleuric membrane becomes the amnion and the outer one, the chorion or serosa. The cavity between the amnion and the embryo is called the amniotic cavity and is lined by ectoderm. Muscle fibres differentiate within the mesoderm of the amnion and the amniotic cavity gets filled with fluid called amniotic fluid. The cavity lying between the amnion and chorion is called the chorionic cavity and is lined by the mesoderm. This chorionic cavity is actually the extra-embryonic coelomic cavity, which is continuous with the coelomic cavity in the embryo proper. The chorion is lined on the outside by the extra embryonic ectoderm. According to Balinsky the formation of the amniotic cavity has a somewhat negative effect as it removes the embryo from the source from where it could obtain oxygen, (Refer Figure 9.3).

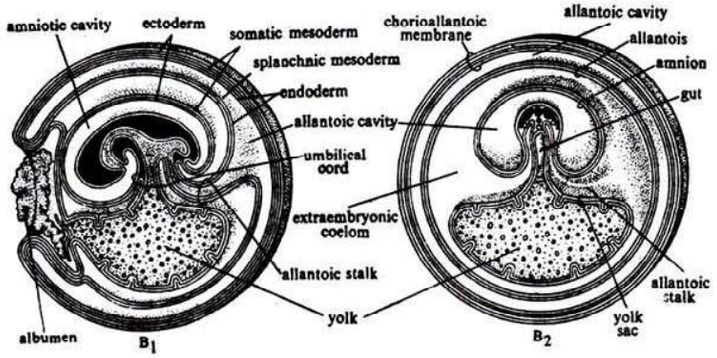

Fig. 9.3 *Formation of Foetal Membrane at Late Stage of Chick Embryo*

- **Development of Allantois:** The allantois first appears late in the 3rd day of incubation. It bulges out as a ventral outgrowth of the endodermal hind

gut and corresponds exactly in nature to the urinary bladder of the amphibians. The outgrowth consists of an inner layer of endoderm and an outer layer of splanchnic mesoderm. The allantois enlarges very rapidly from the fourth day to the tenth day of incubation. It penetrates into the extra-embryonic coelom; into the space between the yolk sac, the amnion and the chorion.

The base of the allantois remains connected with the hindgut of the embryo by means of a narrow allantoic stalk. When the body of the embryo contracts separating the embryo from the extra-embryonic parts, the allantoic stalk and the stalk of the yolk sac remain enclosed together forming an umbilical cord. The distal part of allantois penetrates between the amnion and yolk sac on one side and the chorion on the other side. By the 4^{th} to 10^{th} day of incubation period, the allantois spreads rapidly and completely covers the coelomic space. Soon, the mesodermal layer of the allantois becomes fused with the adjacent mesodermal layer of the chorion to form a single mesodermal layer called chorioallantoic membrane. In the mean-time, the expanding chorioallantois bursts through the vitelline membrane of egg and pushes outward, towards the shell membrane. As does so it progressively envelops the albumen and becomes a sac filled with albumen, called the albumen sac, that helps in the absorption of water and albumen. The chick embryo, through the chorio-allantoic membrane and the shell, takes up about 5 liters of oxygen and gives off about 4 liters of carbon dioxide during its 21 days period before hatching.

On the external surface of the allantois, a network of blood vessels develop and this network is in communication with the embryo proper by means of blood vessels running along the stalk of the allantois and through the umbilical cord. Blood flows to the allantois through the right and left umbilical arteries, that leaves the dorsal aorta at a point which is much more caudal than the starting point of the vitelline arteries. The returning blood flows to the heart through a pair of umbilical veins that originally enter the right and left ducts of Cuvier. Soon, the right umbilical artery and the right umbilical vein disappear and the left umbilical vein develops a new connection. It joins the left hepatic vein and the connection with the duct of Cuvier gets closed. The allantoic circulation functions till the hatching of the chick; when it starts breathing the surrounding air and then the umbilical vessels closes. The allantois dries up and separates from the body of the young chick.

Functions of the Foetal Membranes

The developments of foetal or extra-embryonic membranes are important for those vertebrates that lay their eggs on land. Eggs developing in water, encounter minimum external interference and water provides the egg with various favorable environmental conditions. However, none of the favorable features are provided on dry land where eggs are subjected to desiccation and sudden changes in temperature. The extra-embryonic membranes have thus developed to serve the following functions:

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Functions of Yolk Sac

- The yolk sac which spreads over the large amount of yolk, serves as the digestive and absorptive organ by which the yolk is made available for the growing embryo.
- It functions as the first respiratory organ.
- It acts as a haemopoetic organ like the liver.
- The yolk sac also serves as the place of origin of blood cells, at later stages of development.

Functions of Amnion and Chorion

- The amniotic cavity contains a salty fluid surrounding the embryo. Thus, the embryo can accomplish its development in a fluid medium although it is 'on dry land'. Therefore, the amnion serves as a protective organ where the embryo is saved from the danger of desiccation.
- The amniotic fluid acts as an efficient shock absorber and thus, protects the soft, collapsible and almost skeletons early chick embryo from mechanical shocks.
- As the amnion isolates the embryo from the egg shell, it thus protects it from adhesion to the shell or from friction against it.
- The mesoderm of amnion, during later developmental stages, form muscle cells which contract rhythmically, thus rocking the embryo within the amniotic fluid. This rocking prevents the adhesion of amnion to the different embryonic membranes. It also helps in preventing the stagnation of blood in the vessels, a condition that might tend to occur on account of pressure from growing organs.
- The chorion at later developmental stage joins with the allantois to serve as a nutritional and respiratory organ.

Functions of Allantois

- Allantois acts as a reservoir for the secretions (excretory wastes) coming from the developing excretory organs. During early stages of development the chick excretes mostly urea, but later it becomes chiefly uric acid. This change is significant as urea is a relatively soluble substance and would require large amount of water to keep it at nontoxic level. Uric acid is relatively insoluble and can be stored without any ill effects.
- The chorioallantoic membrane acts as a respiratory surface for the embryo. Thus, the yolk sac, amnion, chorion and allantois can be regarded as an adaptation for the egg and embryo to carry on its development on dry land.

9.3 PLACENTA AND PLACENTATION IN MAMMALS

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Placenta is also known as 'After-birth' structure. Mammals which form placenta for the development of their fetus are referred to as placentalia. The placenta is a materno-foetal temporary organ that develops at the implantation and is required for development of embryo and foetus. Its principal activities are metabolism, respiratory gas exchange, transfer of nutrients, elimination of waste products and endocrine secretion for maintenance of foetus during pregnancy. The placenta is a composite structure produced by the development and apposition of the extra embryonic membranes with the uterine endometrium for the purpose of physiological exchange. In between these two parallel plates, a huge blood sinus, the intervillous space, contains an enormous number of chorionic villi.

Types of Placenta

The placenta consists of two parts:

- Foetal placenta
- Maternal placenta

Foetal Placenta: It is formed by extra embryonic membrane chorion which is the principal component of foetal placenta. It establishes a vascular link between the embryo and the maternal tissues.

Maternal Placenta: Uterine endometrium is a solitary component of maternal placenta. Placenta is also found in diverse groups of animal kingdom such as in *Peripatus, Salpa*, elasmobranchs and certain lizards. In each case, mode of origin and the structure of placenta is different.

Classification of Mammalian Placenta

The mammalian placenta is classified into four different types:

According to Nature of Foetal Membranes Taking Part in Formation of Placenta

- **Chorio-Vitelline or Yolk-Sac Placenta:** It is a primitive type of placenta found in some of the marsupials, for example, Opossum and Kangaroo. In this type of placenta, the allantois remains comparatively tiny and never makes fusion with the chorion, while the yolk sac becomes very huge and combines broadly with the chorion, (Refer Figure 9.4).

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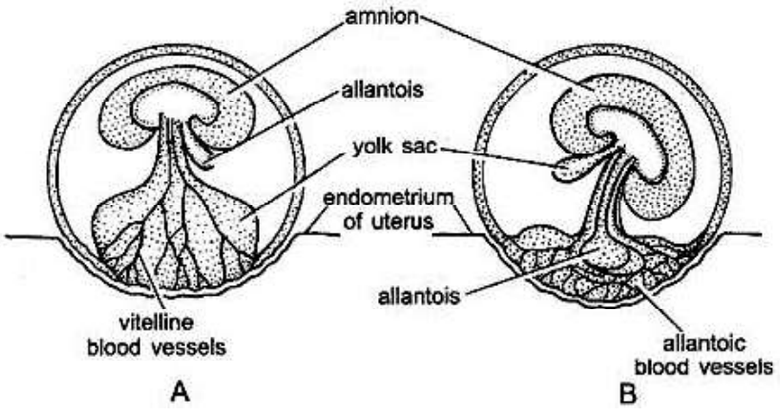

Fig. 9.4 *Placenta in Mammals: Chorio-Vitelline Placenta (A), Chorio-Allntoic Placenta (B)*

- **Chorio-Allantoic Placenta:** In chorio-allantoic placenta, the yolk sac remains undeveloped. The fusion found between the uterine wall and the embryo is lined by chorion and allantois. So, allantois furnishes the chorionic circulation. Since the placenta is formed of chorion and allantois, it is termed as chorio-allantoic placenta, for example., *Parameles, Dasyurus.* etc, (Refer Figure 9.5).

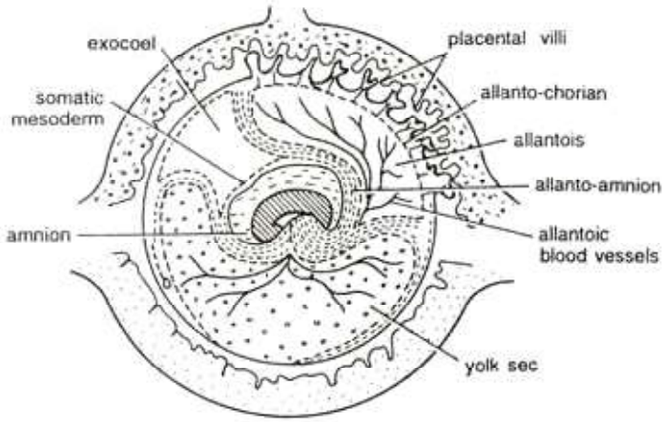

Fig. 9.5 *Eutherian Embryo showing Allantochorionic Villi*

- **Chorionic Placenta:** The chorionic placenta is formed of thickened layer of chorion containing sinuses filled with maternal blood. Chorionic 'villi' containing foetal connective tissue and capillaries expand and cross the chorionic lacunae. Chorionic type of placenta is found in human beings. The portion of the trophoblast which is nearer to the embryo is known as cytotrophoblast. The more external lying part of the trophoblast is called syncytiotrophoblast as it is a syncytium of irregular strands with interstices in between. The part of the uterine wall to which the placenta is joined is termed as decidua basalis. The portion of uterine mucosa and epithelium

which is over the blastocyst forming a capsule, is known as decidua capsularis, the left over part of the uterine wall with which the chorion finally comes in touch, is called as decidua vera.

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According to Degree of Intimacy between Foetal and Maternal Tissue

Five sub-types of placenta in mammals can be distinguished on this basis:

- **Epithelio-Chorial Placenta:** It's the simplest type of placenta where the villi of the chorion dip into the crypts of the uterine wall. For examples: All marsupials, some ungulates and lemurs. Therefore, the molecules of oxygen and other nutrients diffuse from the blood of the mother to that of the embryo through the following: maternal endothelium, maternal connective tissue, maternal uterine epithelium, epithelium of chorion, foetal connective tissues, and foetal endothelium.
- **Syndesmochorial Placenta:** In this type of placenta, the uterine epithelium is eroded. The chorion comes in contact with maternal connective tissue. Thus the nutrients pass through maternal endothelium, maternal connective tissue, chorion, foetal connective tissue and foetal endothelium. This type of placenta is found in ruminant ungulates.
- **Endotheliochorial Placenta:** In this type, uterine epithelium and maternal connective tissues are eroded. Thus the nutritive materials pass through the maternal endothelium, chorion, the foetal connective tissues and foetal endothelium. This condition is found in carnivores.
- **Haemochorical Placenta:** In addition to the uterine epithelium and maternal connective tissues, the maternal endothelium is also eroded. Thus the chorionic villi directly dip in maternal blood. This type of placenta is found in lower rodents, insectivores, bats and man.
- **Haemoendothelial Placenta**: In this type of placenta all the three maternal tissues and two foetal tissues, i.e., chorionic epithelium and chorionic tissue are completely eroded, (Refer Figure 9.6). The foetal blood vessels dip into blood lacunae of the uterus. The number of barriers between the maternal and foetal blood streams, therefore is reduced to just one,for example, higher rodents and rabbit.

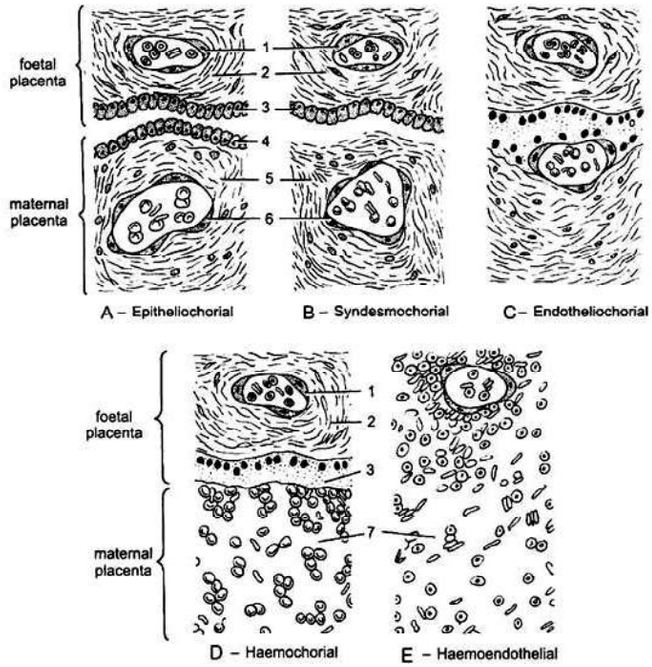

Fig. 9.6 *Placenta in Mammals on the basis of Intimacy of Foetal and Maternal Membranes*

According to degree of contact between chorionic villi and endometrium:

Two sub-types of placenta may be recognized:

- **Non-Deciduate Placenta:** The chorionic villi are simple projections. They have loose connections with crypts in the uterine epithelium. At the time of birth, the chorion peels off from the uterine wall by pulling the villi out of the crypts. So no bleeding occurs at parturition. It is found in pig, cattle, horse and other ruminants.
- **Deciduate Placenta:** In higher Eutherian mammals including dog, rabbit and man, the union between the chorion and the uterine epithelium is much closer. The villi are so closely united with the uterine wall that at parturition, a large part of the uterine tissue is lost along with the foetal membranes. A large amount of bleeding also occurs. As the uterine wall participates in the formation of placenta, such a placenta is called deciduas. The decidua has three types as follows:
- **Decidua Basalis:** The upper part of uterine wall to which the embryo becomes attached is called decidua basalis.
- **Decidua Capsularis:** The part which surrounds the blastocyst and separates it from the cavity of the uterus is called decidua capsularis.
- **Decidua Parietalis:** The parts which form the inner lining of the uterine wall is called decidua parietalis.

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According to Distrubution Pattern of Chorionic Villi

The placenta is classified into four sub types according to the distribution and arrangement of villi on the surface of the chorion, you can also (Refer Figure 9.7).

- **Diffuse Placenta**: The villi are uniformly distributed all over the surface of the blastocyst. It is found in pig, horse and lemurs.
- **Cotyledonary Placenta**: The villi are arranged in patches. Each patch of villi is known as cotyledon. The uterine wall is provided with thickened sockets into which the cotyledons fit, for example, Sheep, cattle and deer.
- **Zonary Placenta**: The villi are arranged in the form of a belt around the middle of the chorionic sac, for example, Cat and dog.
- **Discoidal Placenta**: The villi are restricted to a small disc shaped area of the blastocyst and hence known as discoidal placenta, for example Insectivores, rodents, anthropoid apes and bats.

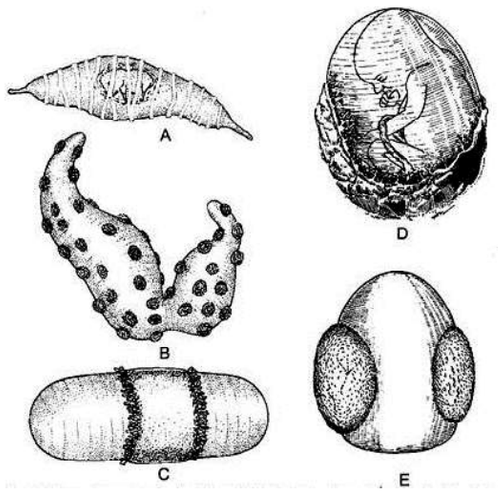

Fig. 9.7 *Diffuse Placenta (A), Cotyledonary Placenta (B), Zonary Placenta (C), Monodiscoidal Placenta of Human (D), and Bidiscoidal Placenta of Monkey (E).*

Physiology and Functions of Placenta

Placenta forms a physiological barrier and a semipermeable membrane between the mother and the foetus. It prevents the straight mixing of the maternal and the foetal blood. It prevents the entry of harmful materials. It allows smaller molecules to diffuse. It provides nourishment and oxygen to the embryo. Oxygen, water and small molecules such as monosaccharides, salts of sodium, potassium and magnesium diffuse from the maternal blood into the foetal blood through the placenta. Macromolecules of polysaccharides, lipids and proteins may be engulfed by trophoblast cells by pinocytosis. The placenta provides immunity to the fetus against certain diseases such as diphtheria, scarlet fever, small pox and measles. The antibodies which have developed in the blood of mother against these diseases

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are passed to the foetal placenta. Similarly Rh antibody also passes through placenta. Blood proteins cannot pass through the placenta because they are large molecules, so they are broken into amino acids and transmitted. The foetus rebuilds complex proteins. The most important function of placenta is the transfer of food stuffs from the mother to the foetus. Many drugs consumed by the mother penetrate the placental barrier and cause most adverse effects in the embryo, for example, thalidomide used as a sedative by women in early pregnancy causes extensive deficiencies. Certain pathogenic organisms and viruses can penetrate through the placenta and infect the foetus if the mother is infected. For example mother is suffering form a pathogenic disease such as AIDS or Syphilis, then the fetus is likely to have the disease. Placenta acilitate the exchange of oxygen and carbon dioxide between fetus and mother thus helps in the process of respiration. Oxygen from the maternal blood diffuses into the fetus blood, where oxygen concentration is comparatively less than maternal blood. Similarly, the diffusion of carbon dioxide from fetus blood into maternal blood stream also takes place.

Check Your Progress

1. What is nuclear transplantation?
2. What is Teratogenesis?
3. Which is the first extra-embryonic membrane to make appearance?
4. What are the blood vessels of area vasculosa joined together by?

9.4 NUCLEAR TRANSPLANTATION

Nuclear cloning, also referred to as nuclear transfer or nuclear transplantation, denotes the introduction of a nucleus from an adult donor cell into an enucleated oocyte to generate a cloned embryo. When transferred to the uterus of a female recipient, this embryo has the potential to grow into an infant that is a clone of the adult donor cell, a process termed "reproductive cloning." Nuclear transplantation is a method in which the nucleus of a donor cell is relocated to a target cell that has had its nucleus removed (enucleated). Nuclear transplantation has allowed experimental embryologists to manipulate the development of an organism and to study the potential of the nucleus to direct development. Nuclear transplantation, as it was first called, was later referred to as somatic nuclear transfer or cloning. Yves Delage first wrote about nuclear transplantation in 1895, speculating that if one were to replace an egg nucleus with another egg's nucleus, full development would occur. Later in 1938, H. Spemann suggested an experiment whereby, using technologies not yet available to him, one could remove the nucleus of an egg and replace it with a different nucleus extracted from a developed cell. Thomas King and Robert Briggs were the first to perform experimental nuclear transplantation. The technique was soon after used by J. Gurdon and eventually led to the first clone of a mammal, 'Dolly' the sheep, by I. Wilmut in 1996.

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Nearly fifteen years after Spemann wrote about the possibility of nuclear transplantation, Briggs and King, using northern leopard frogs (*Rana pipens*), performed the first nuclear transplantation experiment. They transplanted the nucleus from an early stage embryo to an unfertilized egg that had been enucleated. The egg cell was pricked with a clean glass needle in order to induce a fertilization-like response. The faux activation of fertlization allowed for extraction of the nuclear material inside while also activating the host egg cell. Meanwhile, the nucleus of a donor cell was extracted and then inserted into the newly enucleated and activated egg cell. That process induced development of the host egg according to the instructions of the newly inserted nucleus, resulting in the formation of an organism with the same genetic material as the donor cell, or a clone. Briggs and King continued to examine the potential of differentiated cells throughout the 1950s. They found that if the donor nucleus was extracted later in development, the potential of directing full development in the activated egg cell was greatly reduced. After the Briggs and King experiments it was generally accepted that the nuclear material in developing cells slowly loses its potential for full development.

That view was challenged in 1958 when Gurdon's experiments with African claws frogs (*Xenopus levis*) produced fully developed frogs from the transferred nucleus of cells much later in development. Gurdon allowed the cloned frogs to develop to sexual maturity and was then able to mate two sexually mature clones, suggesting that the donor nuclei were able to fully redirect development. Gurdon's experiments were widely accepted by the scientific community but questions remained for several decades. Scientists were concerned about whether the nucleus of the host egg cell was truly enucleated. The question of whether remnants of the host egg cell or the inserted nucleus directed development remained unanswered from 1958 to 2002, despite many attempts by Gurdon to prove it was the inserted nucleus. In 2002, however, K. Hochlelinger and R. Jaenisch published an experiment using nuclear transplantation of mature white blood cells to generate mouse clones. Hochedlinger and Jaenisch were able to show that the inserted nucleus induced development in the host egg cell.

Although experimental embryologists continued to use nuclear transplantation to create clones of several species, Ian Wilmut's cloning experiment in 1996 was a controversial and widely publicized cloning experiment. Dolly was cloned using the nucleus of a mammary gland cell from an adult sheep and transplanting it into an enucleated egg cell from another sheep. The activated egg cell was then transferred into a third surrogate sheep that carried Dolly to term. Dolly died at the age of six due to lung disease and severe arthritis, and although her death was not attributed to the fact that she was a clone, many believe that the relationship between telomeres and ageing was the reason for her demise. Nuclear transplantation may have begun as a subtle idea in the late 19th and early 20th centuries, but it evolved into a feasible and widely used process by experimental embryologists in the late 1990s. The cloning of Dolly the sheep worried many about the possibility of human cloning and the moral boundaries of modern advances

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in science. In the context of the embryonic stem cell discourse of the late 1990s and early twenty-first century, somatic nuclear transfer has been contrived into moral arguments about rights of the human embryo. Furthermore, nuclear transplantation has spurred ethical discussion on the value of a human life during all stages of development. Many scientists have abandoned the methods involved in nuclear transplantation and have adopted methods set forth by S. Yamanaka in his experiments involving induced pluripotent stem cells.

Check Your Progress

5. With what does the base of the allantois remain connected with?
6. What is placenta also known as?

9.5 ORIGIN OF GENE THEORY

The Gene theory is one of the basic principles of biology. The main concept of this theory is that traits are passed from parents to offspring through gene transmission. Genes are located on chromosomes and consist of DNA. They are passed from parent to offspring through reproduction. The principles that govern heredity were introduced by a monk named Gregor J. Mendel in the 1860's. These principles are now called Mendel,s law of segregation and law of independent assortment.

Embryological Origin of Gene Theory

The original support for the gene theory of inheritance came largely from the studies of E. B. Wilson, Theodor Boveri and Thomas Hunt Morgan. Each of these scientists began his career as an embryologist. The emergence of the gene theory out of embryology is seen in the context of researchers' attempts to solve the problem of which cellular compartment, the nucleus or the cytoplasm directed development. Crucial to this transition from embryology to genetics was the discovery of the sex chromosome, a nuclear structure believed to undergo direct sexual development. We shall see that the constant questioning and retesting of the chromosomal theory of sex determination inadvertently formed the basis for Morgan's proof that the genetic factors were physically located on the individual chromosomes. Finally, the research into the chromosomal models of inheritance displays many examples of how the adherence of scientists to older ideas causes them to interpret new data so as not to conflict with previously held assumptions. This conservative tendency is seeing in case of McClung, who insisted on the environmental determination of sex even though he had discovered the mechanism for its intrinsic determination and especially in T. H. Morgan's ten-year refusal to espouse the Mendelian genetics which he would later champion.

Morgan's refusal to accept the Sutton-Boveri synthesis of Mendelism and cytology becomes a chief concern in his essay. His arguments against this view are seen to arise from his previous embryological experiences, which convinced him

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that chemical reactions in the cytoplasm were responsible for development, rather than morphological changes within the nucleus. This view contrasts with other analyses, which relate Morgan's refusal to his 'empirical' attitude, his dislike for theorizing, or to his 'romantic' temperament. Wilson's rapid acceptance of the Sutton-Boveri hypothesis is seen to result from his prior conviction that the nucleus controls development. The reasons for these differences between Morgan and Wilson are traced back to two of Wilson's embryological beliefs which were not shared by Morgan. First, Wilson believed that the cell was the primary unit of development. Morgan had insisted that developmental forces molded the embryo irrespective of cellular boundaries. Secondly, Wilson believed that the development of all organisms was essentially the same. He abolished the distinction between 'mosaic' and 'regulative' egg cleavage, stating that this was merely an artifact of how early the nucleus programmed the cytoplasm. This allowed him not only to accept Morgan's data, but also to extrapolate from unicellular organisms to embryos. Hence, Wilson was able to see the nuclear control of protozoan morphogenesis as an instructive analogue of those processes occurring during embryogenesis.

The embryological origin of the gene theory demonstrates how the biases of one discipline are effectively carried over into a new field. It shows, too, how a relatively small group of investigators pursuing a problem in one area can generate the foundations of an entirely new science. The entry of Wilson and Morgan into genetics will be seen as an attempt to answer fundamental embryological questions and their opposing positions-Wilson's acceptance of the chromosome theory and Morgan's long-standing rejection of it-will be seen in the context of their commitments to certain embryological theories. Other analysis has been made of Morgan's and Wilson's work prior to the gene theory. Garland E. Allen has carefully documented Morgan's disagreements with the chromosomal theory of sex determination, but although he states that this view was typical of other embryologists, he does not relate Morgan's views to their larger embryological context.

Allen constructs his analysis from a cytological-genetic perspective rather than viewing Morgan as a participant in to recent embryological controversies. Morgan's rejection of the Sutton-Boveri hypothesis stemmed from his prior belief in the cytoplasmic control of development. There are two studies which have investigated the embryological researches of Morgan and Wilson as a precondition for their subsequent work. Wagers discusses Morgan's embryological studies, and claims that Morgan became a Mendelian after W. E. Castle's 1909 paper formulating a physiological model for Mendelian inheritance. However, there is much evidence against this interpret.

Adding to Boveri's evidence, demonstration was done between nuclear chromosomes and organisms development: XO or XY embryos became male; XX embryos became female. Here was a nuclear property that correlated with development. Eventually, Morgan began to obtain mutations that correlated with

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sex and with the X chromosome and he began to view the genes as being physically linked to one another on the chromosomes. The embryologist Morgan had shown that nuclear chromosomes are responsible for the development of inherited characters.

Differential Gene Activation

The principle of differential gene activation states that the differences among cells having the same genetic content are the result of different sets of genes being turned on or off. Differential gene activation is the basis of cell differentiation and function. The control of gene activity in higher organisms can take place at more than one level, for example, at the level of DNA, RNA polymerase, or histone. By interacting with DNA, histone determines whether RNA polymerase can ever bind at the respective chromosome region and whether mechanisms operating at other levels can ever come into play. Because the interaction between histone and DNA is ionic, it is primarily influenced by the ionic milieu of the chromosomal environment. Inorganic anions such as chloride or phosphate may affect the histone-DNA interaction as organic ions, such as amino acids, nucleotides, polyamines, or even more complex "ions" such as phosphoprotein, acidic protein, or RNA.

Some studies also showed that some genes also get activated due to action of phytochrome$_{730.}$ Phytochrome-induced photomorphogenesis in the mustard seedling (*Sinupis ulba L.*) which can be regarded as being representative of the dicotyledonous seedlings has been analysed. It has been observed that RNA and protein synthesis have some effects on actinomycin D and puromycin, which support the hypothesis that the 'positive' photoresponses of the seedling can be explained by a differential gene activation through P,30. 'Positive' photoresponses are those which are characterized by an initiation or an increase of biosynthetic or growth processes (for example, biosynthesis of anthocyanin ; growth of cotyledons). The lag-phase of this type of photoresponse is rather long, 'Negative' photoresponses are those which are characterized by an inhibition of growth processes or other physiological processes like translocation. Here the lag-phase is short. Inhibition of hypocotyl lengthening is a typical response of this sort. The concept of differential gene repression may serve as a working hypothesis to approach the causal analysis of phytochromeinduced 'negative' photoresponse.

9.6 TERATOGENESIS

Every year innumerable deaths of infants are due to congenital abnormalities or defects produced in infants or fetus either due to environmental factors or genetic factors or sometimes both. Approximately 3-5% of live births are complicated by a birth defect each year totaling around 120,000 babies. According to the survey done by W.H.O. in 2013 nearly 276,000 new born dies before one month of life every year as a results of congenital abnormalities. This phenomenon of producing congenital abnormalities in an embryo or fetus is termed as teratogenesis.

Teratogenesis is defined as the prenatal toxicity affecting the postnatal life of an organism characterized by the structural and functional defects in the developing embryo or fetus. Due to this, malformed or deformed babies are produced usually with short life span. Extrinsic factors responsible for producing these birth defects and deformities are known as teratogens. The word teratogen derived from Greek word *teratos* means monster. Exposure to the teratogen affects the fetus or embryo in a variety of ways, such as the duration of exposure, the amount of teratogenic substance and the stage of development the embryo or fetus during the exposure. Teratogens may affect the embryo or fetus in a number of ways, causing physical malformations, problems in the behavioral or emotional development of the child and decreased Intellectual Quotient (IQ) in the child. Additionally, teratogens may also affect pregnancies and cause complications such as preterm labors, spontaneous abortions or miscarriages.

Human development is divided into two phases Embryonic period (period lasts till 8th week of pregnancy and involves organogenesis, i.e., formation of organs) and Fetal period (from 9th week till parturition and involves growth and modeling). Utmost teratogens produce their effects during the crucial period of development i.e., embryonic period. Fetus is more susceptible to teratogens from week 3 to week 8 of the gestation period as most of the organs are formed during this period, however nervous system remains susceptible throughout the gestation period as it is continuously formed throughout the gestation period. Prior to 3 weeks of the pregnancy, exposure to teratogens does not produce any congenital abnormalities as the encountered teratogens at this time either damages most or all the cells of embryo, resulting in death or it kills few cells only thereby allowing the embryo to recover.

Factors Involved in Teratogenesis

There are various factors involved in teratogenesis such as alcohol, retinoic acid, endocrine disruptors, BisphenolA (BPA), pesticides, heavy metals, viruses, pathogens, etc.

Alcohol: Alcohol or ethanol is one of the most devastating teratogen. Consumption of alcohol during first and second week of pregnancy is more dangerous as it is responsible for the development of Fetal Alcohol Syndrome (FAS). Baby born with FAS is characterized by small head size, indistinct philtrum (Philtrum=pair of ridges runs between nose and mouth above centre of upper lip), narrow vermillion border, low nose ridge and brain of such child is also dramatically smaller, shows poor development due to lack of neurons and neuroglias, (Refer Figure 9.8). FAS is most prevalent type of mental retardation syndrome and mostly occurs due to consumption of alcohol during pregnancy, occurring approximately 1 out of 650 children in US states.

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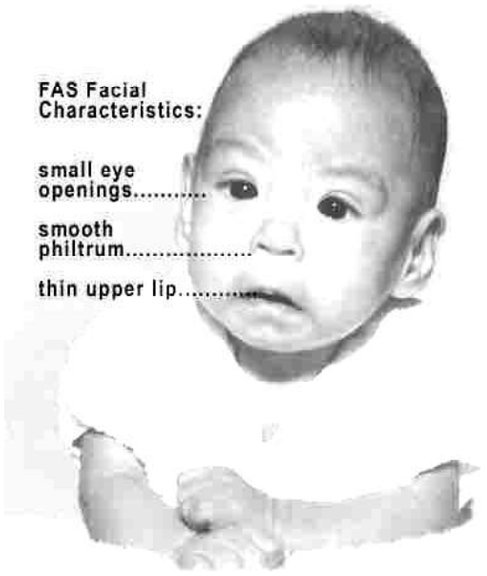

Fig. 9.8 *Baby with Fetal Alcohol Syndrome*

Another syndrome that is caused by the consumption of alcohol is known as Fetal Alcohol Spectrum Disorder (FASD) and this is coined to encompass all the alcohol induced malformations, functional defects and is mostly associated with high level consumption of alcohol. Consumption of alcohol not only produces birth defects in human babies but also in case of other mammals such as mice. Studies has shown that at the time of gastrulation if mice embryo is exposed to alcohol then the alcohol induces defects in face and brain whereas the same treatment given to human fetus incite the defects in which nose and upper lip were poorly developed along with incomplete development of forebrain. Ethanol or alcohol acts as teratogenic factor as it intervene in some fundamental processes such as cell migration, cell proliferation, cell adhesion and survival. In some studies it has been observed that in Alcohol-Exposed Embryos, Expression of Sonic Hedgehog Gene is down regulated (Shh) that helps in establishment of facial midline structures, (Refer Table 9.1).

Table. 9.1 *Some Agents thought to Cause Disruptions in Human Fetal Development*

Drugs and chemicals	Alcohol, Aminoglycosides, (Gentamycin), Aminopterin, Antithyroid agents (PTU), Bromine, Cortisone, Diethylstilbesterol (DES), Diphenylhydantoin, Heroin, Lead, Methylmercury, Penicillamine, Retinoic acid, (Isotretinoin, Accutane), Streptomycin, Tetracycline, Thalidomide Trimethadione, Valproic acid, Warfarin
Ionizing radiation	X-Rays
Disease	Hyperthermia (Fever)
Infectious Microorganisms	Coxsackie virus, Cytomegalovirus, Herpes simplex, Parvovirus, Rubella (German measles), *Toxoplasma gondii,* (toxoplasmosis), *Treponema pallidum* (syphilis)
Metabolic conditions In the mother	Autoimmune disease, (Autoimmune disease (including Rh incompatibility), Diabetes, Dietary deficiencies, malnutrition

Metabolic Conditions in the Mother: Autoimmune disease, (Autoimmune disease (including Rhincompatibility), Diabetes, Dietary deficiencies, malnutrition.

Retinoic Acid: Retinoic acid is a Vitamin A derivative that plays a role in specifying Anterior-Posterior axis and formation of Jaws and hearts during the development of mammalian embryo, (Refer Figure 9.9). For medication purposes retinoic acid is usually given to treat Severe Cystic Acne in form of Accutane but in 1950 it has been disclosed that retinoic acid has deleterious effects on pregnant ladies as it responsible for the development of various abnormalities or birth defects such as absent or presence of defective ears, absent or small jaws, defective cleft palate or aortic arch and abnormalities of central nervous system. All these abnormalities are occurred due to failure of migration of neural crest cells into pharyngeal arch thus are not able to form ear and jaws.

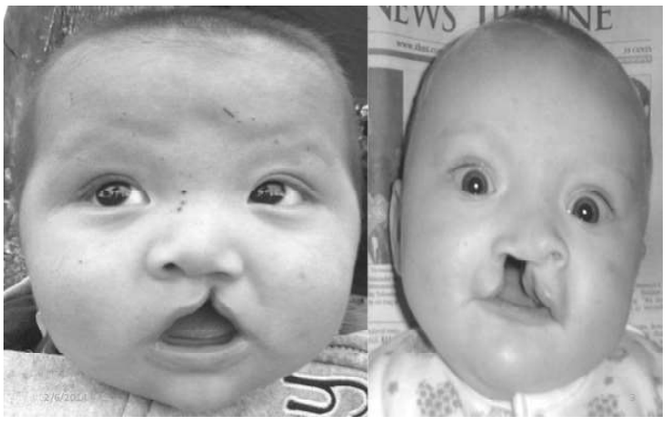

Fig. 9.9 *Cleft Lip and Cleft Palate in Baby*

Bisphenol-A: Bisphenol-A is a chemical produced in large quantities for use primarily production of polycarbonate plastics and epoxy resins. Polycarbonate plastics have many applications including use in some food and drinks packaging, for example, water and infant bottles, compact discs and medical devices. Epoxy resins are used a lacquers to coat metal products such as food cans, bottle tops and water supply pipes. The primary source of exposure to BPA for most of pregnant ladies is through diet, food, beverages, dust ,water, air, etc. BPA act as teratogenic as it is responsible for bringing out deformities in infant such as premature puberty ,early sexual maturation, low sperm count, prostate enlargement etc. So this has been proved that teratogens not only cause defects in the embryo or fetus but also has impact on the post natal life, i.e., life after birth.

Drugs: In the 1962, Carson disclosed that pesticide DDT is responsible for the destruction of Bird eggs and also preventing reproduction in several species. Another drug Thalidomide a sedative used in managing pregnancy could cause limb or ear abnormalities in human fetus.

Heavy Metals: Heavy metals such as zinc, mercury and lead are powerful teratogens and the industrial pollution has resulted in the high concentrations of

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heavy metals. Mercury and Lead damages the developing nervous system and Hg is preferentially absorbed by regions of developing cerebral cortex .In one study it has been observed that pregnant mice were given Hg on the 9th day of gestation period and babies were born with small brain and small eyes.

Viruses: Herpes simplex virus (*Treponema palladium),* Cytomegalovirus both act as teratogens results in the deafness, blindness and mental retardation to late embryo where as completely fatal to early embryo. In 1964 when epidemicity of Rubella (German Measles) occurs in United States, More than 20,000 fetus infected by Rubella were born blind ,deaf or both ,along with this fetus has heart defects as well as mental retardation.

Check Your Progress

7. What does placenta form?
8. What is placenta?
9. What is teratogenesis?
10. What has resulted to the high concentrations of heavy metals?

9.7 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Nuclear transplantation is a method in which the nucleus of a donor cell is relocated to a target cell that has had its nucleus removed (enucleated).
2. Teratogenesis is a prenatal toxicity characterized by structural or functional defects in the developing embryo or fetus.
3. The yolk sac is the first extra-embryonic membrane to make its appearance.
4. All the blood vessels of area vasculosa communicate with each other and are joined together on the periphery by the terminal sinus
5. The base of the allantois remains connected with the hindgut of the embryo by means of a narrow allantoic stalk.
6. Placenta is also known as 'After-birth' structure.
7. Placenta forms a physiological barrier and a semipermeable membrane between the mother and the foetus.
8. The placenta is a composite structure produced by the development and apposition of the extra embryonic membranes with the uterine endometrium for the purpose of physiological exchange.
9. Teratogenesis is defined as the prenatal toxicity affecting the postnatal life of an organism characterized by the structural and functional defects in the developing embryo or fetus.
10. The industrial pollution has resulted in the high concentrations of heavy metals.

9.8 SUMMARY

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- At the development of chick and other vertebrates, certain specialized embryonic tissues or structures are produced that temporarily or permanently do not enter into the formation of the embryo themselves. These structures are collectively termed as extra-embryonic membranes or foetal membranes or extra-embryonic sacs.
- During the development of chick and other vertebrates, certain specialized embryonic tissues or structures are produced that temporarily or permanently do not enter into the formation of the embryo themselves.
- The extra-embryonic membranes have developed to make the eggs capable of developing on dry land.
- Teratogenesis is a prenatal toxicity characterized by structural or functional defects in the developing embryo or fetus.
- The amnion and chorion are made up of extra-embryonic ectoderm and somatic layer of mesoderm, while the yolk sac and allantois are composed of extra-embryonic endoderm and splanchnic layer of mesoderm.
- The yolk sac is the first extra-embryonic membrane to make its appearance.
- The amnion and chorion are developed simultaneously and both are derived from the extra-embryonic somatopleure.
- Placenta is also known as 'After-birth' structure. Mammals which form placenta for the development of their fetus are referred to as placentalia.
- The placenta is a composite structure produced by the development and apposition of the extra embryonic membranes with the uterine endometrium for the purpose of physiological exchange.
- Placenta forms a physiological barrier and a semipermeable membrane between the mother and the foetus.
- The antibodies which have developed in the blood of mother against these diseases are passed to the foetal placenta. Similarly Rh antibody also passes through placenta.
- Nuclear cloning, also referred to as nuclear transfer or nuclear transplantation, denotes the introduction of a nucleus from an adult donor cell into an enucleated oocyte to generate a cloned embryo.
- Nearly fifteen years after Spemann wrote about the possibility of nuclear transplantation, Briggs and King, using northern leopard frogs (Rana pipens), performed the first nuclear transplantation experiment.
- The Gene theory is one of the basic principles of biology. The main concept of this theory is that traits are passed from parents to offspring through gene transmission.

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- The embryological origin of the gene theory demonstrates how the biases of one discipline are effectively carried over into a new field.
- Allen constructs his analysis from a cytological-genetic perspective rather than viewing Morgan as a participant in to recent embryological controversies.
- The principle of differential gene activation states that the differences among cells having the same genetic content are the result of different sets of genes being turned on or off.
- Retinoic acid is a Vitamin A derivative that plays a role in specifying Anterior-Posterior axis and formation of Jaws and hearts during the development of mammalian embryo.
- Bisphenol-A is a chemical produced in large quantities for use primarily production of polycarbonate plastics and epoxy resins.
- Heavy metals such as zinc, mercury and lead are powerful teratogens and the industrial pollution has resulted in the high concentrations of heavy metals.
- The extra-embryonic membranes have developed to make the eggs capable of developing on dry land. The eggs of reptiles, birds and prototherian mammals have a protective shell around it.
- Extra-embryonic membranes are the membranes formed of embryonic tissues, which extend out and beyond the strict confines of the embryonic body and are adapted to fulfill the care and maintenance of the developing embryo.
- Chorion is a very thin membrane and it covers the embryo and other extra-embryonic membranes. Allantois serves as an excretory and respiratory structure. It is a large sac like structure in reptiles and birds, while its role in mammals varies with the efficiency of the interchange that takes place at the foetal-maternal interface.
- The amnion and chorion are made up of extra-embryonic ectoderm and somatic layer of mesoderm, while the yolk sac and allantois are composed of extra-embryonic endoderm and splanchnic layer of mesoderm.
- Nuclear transplantation is a method in which the nucleus of a donor cell is relocated to a target cell that has had its nucleus removed (enucleated). Nuclear transplantation, as it was first called, was later referred to as somatic nuclear transfer or cloning.
- The Gene theory is one of the basic principles of biology. The main concept of this theory is that traits are passed from parents to offspring through gene transmission. Genes are located on chromosomes and consist of DNA.
- The phenomenon of producing congenital abnormalities in an embryo or fetus is termed as teratogenesis.

- Teratogenesis is defined as the prenatal toxicity affecting the postnatal life of an organism characterized by the structural and functional defects in the developing embryo or fetus.
- Extrinsic factors responsible for producing these birth defects and deformities are known as teratogens.

9.9 KEY WORDS

- **Yolk sac:** It is the most primitive structure containing network of blood vessels and encloses the yolk of the egg.
- **Amnion:** The amnion is a thin membrane which eventually encloses the entire developing embryo in a fluid-filled sac.
- **Chorion (Serosa):** It is a very thin membrane and it covers the embryo and other extra-embryonic membranes.
- **Allantois:** It serves as an excretory and respiratory structure. It is a large sac like structure in reptiles and birds, while its role in mammals varies with the efficiency of the interchange that takes place at the foetal-maternal interface.

9.10 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short Answer Questions

1. Which type of placenta is present in ruminant ungulates?
2. Can viruses acts as teratogenic agents? Give examples.
3. Give an example of some teratogenic agents.
4. What is philtrum? In which condition does it gets indistinct?
5. Which extra embryonic membrane acts as liver?

Long Answer Questions

1. Explain in detail about the factors affecting teratogenesis.
2. Write a note on origin of gene theory.
3. Placenta is an intimate connection between mother and fetus explains why?
4. What are the functions of different fetal membranes in chick and why they are important?
5. Give the classification of different types of placenta with examples.

9.11 FURTHER READINGS

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UNIT 10 ASSISTED REPRODUCTIVE TECHNOLOGY

NOTES

Structure

10.0 INTRODUCTION

Assisted Reproductive Technology (ART) is used to treat infertility. It includes fertility treatments that handle both a woman's egg and a man's sperm. It works by removing eggs from a woman's body. The eggs are then mixed with sperm to make embryos. The embryos are then put back in the woman's body. In-Vitro Fertilization (IVF) is the most common and effective type of ART. ART procedures sometimes use donor eggs, donor sperm, or previously frozen embryos. It may also involve a surrogate or gestational carrier. A surrogate is a woman who becomes pregnant with sperm from the male partner of the couple. A gestational carrier becomes pregnant with an egg from the female partner and the sperm from the male partner. The most common complication of ART is a multiple pregnancy. It can be prevented or minimized by limiting the number of embryos that are put into the woman's body. ART refers to treatments and procedures that aim to achieve pregnancy. These (IUI, IVF, TPART) complex procedures may be an option for people who have already gone through various infertility treatment options but who still have not achieved pregnancy. Those interested in ART should discuss the options with their health care provider and may need to consult a fertility specialist.

In this unit, you will study the concept of Assisted Reproductive Technology (ART), monitoring of ovulation phase, super-ovulation and cryopreservation in detail.

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10.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand the Assisted Reproductive Technology (ART)
- Explain the monitoring of ovulation phase and superovulation
- Learn about cryopreservation
- Discuss the advantages of ZIFT

10.2 ASSISTED REPRODUCTIVE TECHNOLOGY (ART)

Assisted Reproductive Technology (ART) is used to treat infertility. It includes fertility treatments that handle both a woman's egg and a man's sperm. It works by removing eggs from a woman's body. The eggs are then mixed with sperm to make embryos. The embryos are then put back in the woman's body. In-Vitro Fertilization (IVF) is the most common and effective type of ART. ART procedures sometimes use donor eggs, donor sperm, or previously frozen embryos. It may also involve a surrogate or gestational carrier. A surrogate is a woman who becomes pregnant with sperm from the male partner of the couple. A gestational carrier becomes pregnant with an egg from the female partner and the sperm from the male partner. The most common complication of ART is a multiple pregnancy. It can be prevented or minimized by limiting the number of embryos that are put into the woman's body. ART refers to treatments and procedures that aim to achieve pregnancy. These (IUI, IVF, TPART) complex procedures may be an option for people who have already gone through various infertility treatment options but who still have not achieved pregnancy. Those interested in ART should discuss the options with their health care provider and may need to consult a fertility specialist.

In this unit, you will study the concept of Assisted Reproductive Technology (ART), monitoring of ovulation phase, super-ovulation and cryopreservation in detail.

In vitro fertilization (IVF) is a complex series of procedures used to treat fertility or genetic problems and assist with the conception of a child. During IVF, mature eggs are collected (retrieved) from your ovaries and fertilized by sperm in a lab. Then the fertilized egg (embryo) or eggs are implanted in your uterus. One cycle of IVF takes about two weeks. IVF is the most effective form of assisted reproductive technology. The procedure can be done using your own eggs and your partner's sperm. Or IVF may involve eggs, sperm or embryos from a known or anonymous donor. In some cases, a gestational carrier, woman who has an embryo implanted in her uterus might be used. Intra Cytoplasmic Sperm Injection (ICSI) is a variant in IVF in which a single sperm is injected into each egg. It is done for semen abnormalities and couples who have failed fertilization. Gamete

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Intra Fallopian Transfer (GIFT) was commonly done in the past, particularly for women who wanted to do IVF but avoid conception outside the body. The eggs are stimulated and harvested just like in IVF, but the eggs and sperm are placed into the fallopian tube where fertilization happens. With the increasing success rate of IVF, GIFT is rarely done nowadays. Zygote Intra Fallopian Transfer (ZIFT) is also similar to IVF but involves transfer of the fertilized egg (the zygote) into the fallopian tube at the time of laparoscopy. In the past, like GIFT, ZIFT had a higher pregnancy rate than IVF. With the advent of improved embryo transfer techniques and better laboratories, ZIFT has also become obsolete. Tubal Embryo Transfer (TET) refers to the same procedure as ZIFT but with transfer done at a later date.

Ovulation cycle tracking is a good first step towards maximizing your chance of conceiving naturally. With ovulation cycle monitoring, there's no need for invasive fertility treatments, medication or surgery. Ovulation tracking is a simple process that helps you identify your most fertile days of each month. This will help you plan intercourse and hopefully increase your chances of conception. When trying to fall pregnant, timing is everything, and many couples get it wrong. Trying to coordinate intercourse with the precise time of ovulation means you'll most likely miss your fertile window and that month's chance to conceive. The most fertile time in your menstrual cycle is your fertile window. It is the few days leading up to ovulation, just before the egg is released from the ovary. After ovulation, the egg survives for just 24 hours, while sperm retain fertilizing capability for two to three days in the fallopian tubes. So we recommend having sex every two to three days throughout your fertile window, and in the lead-up to ovulation. This means sperm are ready and waiting for the egg when you ovulate. Even if you have irregular periods we can help you understand more accurately when yours fertile window is, simply by tracking your ovulation cycle. Ovulation is triggered by a surge of hormones from the pituitary gland, known as the Luteinizing Hormone (LH). This surge normally takes place 24 hours before ovulation, and simple ultrasound scans combined with blood and urine tests can predict and then detect ovulation. This highlights the best time to try and conceive.

Superovulation is a term used to describe the drug-induced production of multiple eggs for use in assisted reproductive technologies such as in IVF. Normally, a woman ovulates just one egg per cycle. With the use of fertility drugs, she may be able to produce several eggs, which can then be retrieved from the ovaries prior to ovulation. Superovulation shouldn't be confused with *ovulation induction*. Clomid is a commonly used ovulation induction medication. During ovulation induction, the goal is for the ovaries to mature only one or two eggs. With superovulation, more than two eggs are desired. Superovulation is also sometimes used during IUI treatment. However, due to the risks of a multiple pregnancies, IUI treatment typically involves ovulation induction. Risks of superovulation include ovarian hyperstimulation syndrome, ovarian torsion, and multiple (twins, triplets, etc.) pregnancy There are also potential risks and side effects related to what kind of treatment is being used (IVF or IUI), as well as risks to the fertility drugs chosen.

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Cryopreservation is the use of very low temperatures to preserve structurally intact living cells and tissues. Unprotected freezing is normally lethal and this chapter seeks to analyze some of the mechanisms involved and to show how cooling can be used to produce stable conditions that preserve life. Cryopreservation is the process of freezing biological material at extreme temperatures; most common -196°C/-321°F in liquid nitrogen (N_2). At these low temperatures, all biological activity stops, including the biochemical reactions that lead to cell death and DNA degradation. This preservation method in theory makes it possible to store living cells as well as other biological material unchanged for centuries. The challenge of cryopreservation is to help cells to survive both cooling to extreme temperatures and thawing back to physiological conditions. Intracellular ice formation in particular is a critical issue that has to be controlled to keep the cell membrane intact and the cells alive. The crucial elements to prevent this are the freezing rate (degrees per minute) and the composition of the freezing medium used. The freezing medium generally consists of a diluter, (sometimes) a protein source, as well as a cryoprotectant compound. The choice of most suitable cryoprotectant will influence the preservation result and will be different between different cells and different species. Cryopreservation technology is important in breeding programs to preserve desired genes, but also provides an opportunity to save endangered species.

Infertility is the central issue in the lives of individuals who suffers from it. It is a source of social and psychological suffering for both men and women within the couple. Interest of people in Assisted Reproductive Technology (ART) has remained high since the birth of the world's first in vitro fertilization baby, Louise Brown, in the United Kingdom. ART allows scientists to manipulate the fertilization process in order to bypass some pathological obstacles such as blocked fallopian tubes and non-functioning ovaries in case of females and blocked vas deferens and low sperm count in males, (Refer Figure 10.1). The birth of Louise Brown in 1978 was the culmination of decades of scientific research in reproductive medicine. Since then, an abundance of breakthroughs in both clinical medicine and basic science have allowed increasing numbers of infertile couples the chance to have a baby. Till now, more than 2 million babies have been born worldwide through Assisted Reproductive Technologies (ART).

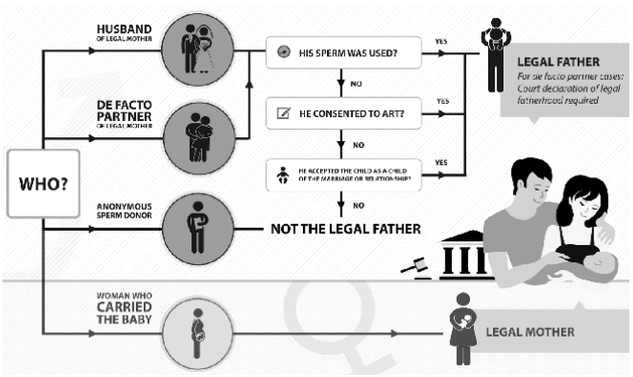

Fig. 10.1 *Schematic Flow Chart of Assisted Reproductive Technology (ART).*

Assisted Reproductive Technologies (ART) are a collection of different techniques designed to help infertile couples to achieve a successful pregnancy, (Refer Figure 10.2). The most popular technology currently in use is in vitro fertilization (IVF), but others include Gamete Intra Fallopian Transfer (GIFT), Zygote Intra Fallopian Transfer (ZIFT), Intra Uterine Insemination (IUI), and Intra Cytoplasmic Sperm Injection (ICSI). Although not encompassed under the umbrella of ART, there are also various hormonal medications that can induce ovulation such as clomiphene citrate that can either be used alone to help women conceive or used in conjunction with the above techniques. Infertility is a problem that has affected people throughout the history, but it was only in the last half of the twentieth century that medical research developed technologies to help those who are infertile to become pregnant.

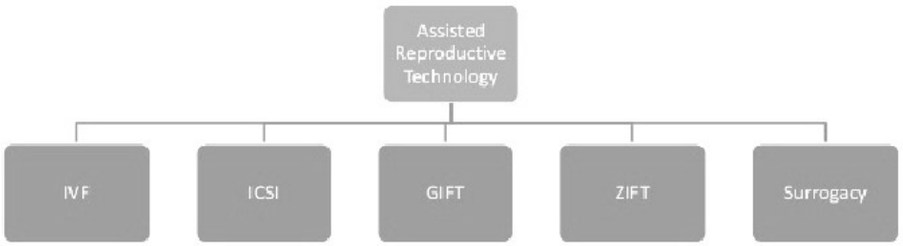

Fig. 10.2 *Methods of ART*

Fertility drugs alone, such as clomiphene citrate, are usually tried first if anovulation (the lack of ovulation) seems to be the only cause of infertility. This technique, if suitable, is the least costly method. Drugs like clomiphene citrate stimulate ovulation by increasing both Follicle Cell Stimulating Hormone (FSH) and Luteinizing Hormone (LH). The physician first administers the lowest daily dosage of 50mg to the patient for five days. If ovulation does not occur, the dosage is raised 50mg at a time until the minimum dosage required to induce ovulation is reached (with a maximum recommended dosage of 250mg). Once ovulation occurs, the physician instructs the patient to have intercourse every other day for a week. If pregnancy does not result, the physician will try again for four to six treatment cycles before turning to ART.

Assisted Reproductive Technology (ART) refers to all technology where gametes are manipulated outside the body. It does not include where only spermatozoa are manipulated like Intra Uterine Insemination (IUI). The first and the most common procedure is In-Vitro Fertilization (IVF), but there is an ever increasing list. The various procedures are as follows: Intra Uterine Insemination (IUI), In-Vitro Fertilization and Embryo Transfer (IVF & ET), Gamete Intra Fallopian Transfer (GIFT), Pronucleate or Zygote Intra-Fallopian Transfer (PROT, ZIFT), Intracytoplasmic Sperm Injection (ICSI), Round Spermatid Nucleus Injection (ROSNI) or Spermatid Injection, and Assisted Hatching.

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10.2.1 Intra Uterine Insemination (IUI)

Intra Uterine Insemination (IUI) is considered one of the oldest techniques to treat infertility and dates back to the early 1900s. The current method may include the physician giving the woman fertility medication to induce ovulation, (Refer Figure 10.3). Once ovulation occurs, naturally or aided by drugs, the physician places collected sperm directly into the uterus, bypassing the cervical mucus. The sperm is either collected fresh from a partner or donor or taken from sperm banks. IUI is generally used if a woman has cervical mucus that is hostile to sperm or if the male has a low sperm count, in which case his semen can be removed to concentrate the sperm. Although IUI is a fairly simple and low-cost technique, it is not commonly used now due to the increased availability of other techniques that have much higher successful fertilization rate and live birth rate, such as IVF, GIFT, and ZIFT. Thus during IUI, the seminal fluid is prepared in the lab (washed with special media), injected inside the uterus after stimulating the ovaries to produce more eggs per cycle.

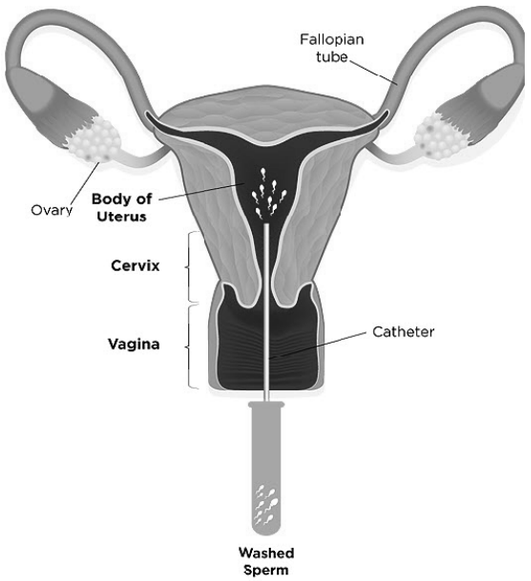

Fig. 10.3 *Diagram showing Intra Uterine Insemination (IUI)*

10.2.2 In-Vitro Fertilization (IVF)

IVF was pioneered in the 1970s by Dr Robert Edwards and Dr. Patrick Steptoe. As of 2009, it is the most commonly used and most successful technique to help women conceive, especially following the development of ICSI by Dr. Gianpiero D. Palermo in 1993. For IVF, the physician first treats the patient with a fertility medication such as clomiphene citrate to stimulate ovulation of several ova, (Refer Figure 10.4). Once the ova are mature, the physician extracts several of them through transvaginal oocyte retrieval. During this procedure, the physician uses a sonogram to guide a needle through the cervix, pierce the vaginal wall, and enter

the ovaries to extract the ova. The sperm used in the procedure is obtained either by masturbation, by a collection condom, or surgically through an incision in the testes if a blockage is preventing the normal ejaculation of sperm. Once the egg and sperm are collected, the physician fertilizes a few of the ova using ICSI; using a needle to manually inject one sperm into an ovum to fertilize it. Once successful fertilization takes place and successful cell division occurs, anywhere from one fertilized egg to several are placed into the uterus for implantation. The number of fertilized eggs placed in the uterus depends on a woman's age as well as other factors that may affect the chance of at least one egg implanting. Since often more than one egg is placed in the uterus, the likelihood of more than one implanting is also relatively high, bringing the possibility of multiple births.

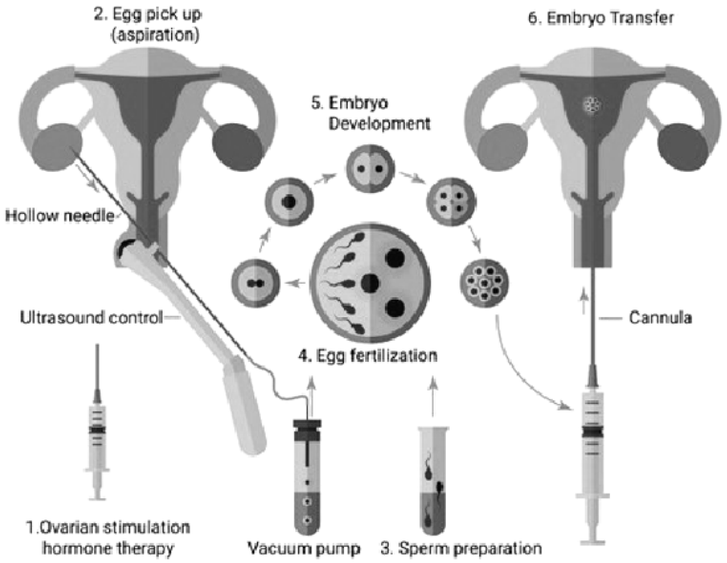

Fig. 10.4 *Showing In-Vitro Fertilization (IVF)*

10.2.3 Gamete Intra Fallopian Transfer (GIFT)

GIFT is a procedure initially developed by Dr. Ricardo Asch in the 1980s. In this technique the physician extracts the ova and sperm with the same procedures as in IVF, but the ova are not fertilized in vitro (outside of the body). Instead, the collected ova and sperm are not mixed until the physician inserts each into the fallopian tubes where they come into contact and allow fertilization, (Refer Figure 10.5). The likelihood of successful fertilization leading to a pregnancy is lower with GIFT than with IVF or ZIFT, but some patients prefer this method because it costs less and is considered a more 'natural' method since fertilization occurs inside the body as it would normally. Thus in GIFT, the sperms and eggs are placed in fallopian tube to allow fertilization in natural site. So that woman must have at least one normal open fallopian tube.

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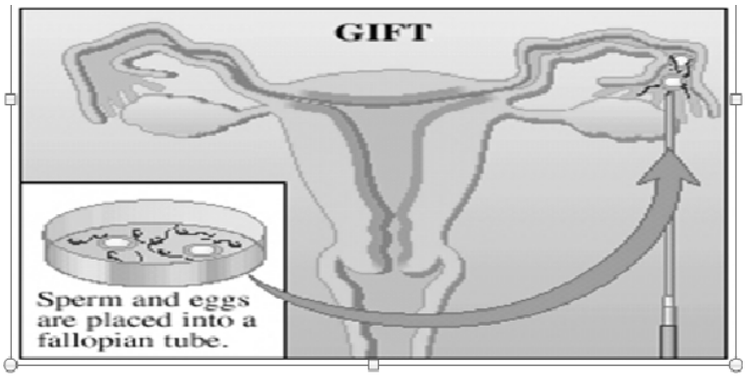

Fig. 10.5 *Showing Gamete Intra Fallopian Transfer (GIFT)*

Gamete Intra Fallopian Transfer (GIFT) has emerged as one of the major Assisted Reproductive Technologies (ART). It began in 1979 with a case report in which clomiphene citrate was given to a woman on cycle days 5–9 and artificial insemination performed on cycle day 12. A laparotomy was performed the following morning to reanastomose the ligated fallopian tubes. Six follicles were aspirated and the follicular fluid divided equally and transferred into each reopened tube. The first successful transfer of both sperm and oocytes was reported in 1983 in six patients with a history of Pelvic Inflammatory Disease (PID). As in the past, it still requires less laboratory equipment and less complexity than IVF and therefore remains an important procedure for a specific category of patients.

GIFT was developed out of a desire to place gametes directly into their natural physiologic environment in order to enhance the potential for fertilization. It is not a procedure that can be used for all patients because at least one patent fallopian tube is required and severe oligospermia is a relative condition. In general, success rates for IVF and GIFT are comparable. Because GIFT requires general anesthesia and a laparoscopy in most instances, most centers prefer to focus the majority of their cases on IVF to reduce operative risk, time and recovery, and to verify fertilization.

As a result of these considerations, GIFT is now used only in niche situations, and it is likely that, over time, GIFT will become an even smaller percentage of ART. Nevertheless, GIFT will continue to be an important option for those individuals who either for personal or religious reasons are opposed to IVF and for those centers that cannot afford or do not have the laboratory equipment, space and technical expertise needed to perform IVF. Various techniques constitute assisted reproduction, one of which is Gamete Intra Fallopian Transfer (GIFT). The first example of GIFT involved primates during the 1970s, however, the technology was unsuccessful until 1984 when an effective GIFT method was invented by Dr R. Asch at the University of Texas Health Sciences Center and the procedure resulted in the first human pregnancy. The GIFT technique was created in hopes of generating an artificial insemination process that mimicked the physiological sequences of normal conception. The technique was further advanced

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at the Center for Reproductive Health at the University of California, Irvine, when Asch and his associate Jose Balmaceda employed a newly developed catheter into the GIFT procedure that eliminated the need for general anesthesia in the later stages of the procedure.

On average, the GIFT cycle takes four to six weeks before fertilization occurs. Women undergoing GIFT begin the procedure with hormonal treatments similar to patients undergoing In-Vitro Fertilization (IVF). The hormonal treatments are administered in order to promote the development of oocytes, which are the precursor to eggs stored in the ovaries. For the highest chance of success with GIFT, fully mature eggs are essential. The male's sperm is also manipulated in order to promote sperm capacitation so it is primed to fertilize the egg. Once the sperm is capacitated, 100,000 to 500,000 motile sperm are utilized in the GIFT procedure. For the highest chance of success with GIFT, an estimated 1.5 million sperm should be motile with at least thirty percent having normal morphology.

The GIFT process begins by obtaining the father's semen two hours before the mother undergoes a laparoscopic procedure to harvest her eggs. A small incision is made near the woman's navel and her eggs are harvested with the use of a fiber-optic viewing device known as a laparoscope. Once the sperm and eggs are collected from both parents, they are immediately placed in the woman's fallopian tubes through a catheter. The catheter contents are separated by air to prevent fertilization prior to the transfer. Depending on the patient's age and the maturity of the oocytes, two to five oocytes are transferred into the fallopian tubes along with the sperm. The transfer of multiple oocyctes carries the possibility of multiple pregnancies, which occurs in an estimated thirty percent of assisted reproductive pregnancies. After the sperm and oocytes are delivered to the woman's fallopian tubes and consequently mixed, the hope is that the resulting embryo or embryos will divide normally, move down to the uterus to implant, and result in a healthy live birth.

The GIFT procedure is considered to be very similar to the process of normal conception since fertilization occurs within the woman's body. Because the GIFT procedure closely resembles natural or unassisted reproduction, it is one of the few reproductive technologies approved by the Vatican; no decisions are made as to which embryos are implanted or discarded, the embryo itself is not manipulated, and fertilization occurs naturally in vivo rather than artificially in vitro. However, one point of contention with Catholic doctrine results from obtaining sperm through masturbation. Gift is an available treatment for infertility caused certain ovary disorders, endometriosis and cervical problems, but it does not treat women with untreated fallopian tube blockages. GIFT requires at least one healthy fallopian tube, whereas treatments such as in vitro fertilization do not. Results of GIFT vary depending on the age of the patients and the quality of the sperm. Women have decreased fertility odds and increased miscarriage risks with increasing age and most successful cases are with women having thirty-five years of age or younger. The GIFT technique is generally more expensive and more

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invasive than IVF because the former requires surgical procedures. According to the 2004 report from the Center for Disease Control and Prevention on Assisted Reproductive Technology, GIFT is the least selected technique with only one percent of 94,242 couples undergoing the procedure. Of the one percent of couples undergoing a GIFT procedure, twenty-three percent result in a live birth.

Although GIFT is seldom chosen among the different assisted reproduction techniques, it remains an option for treating infertility. GIFT is one artificial insemination technique that is accepted by the Vatican, making this technology an appropriate choice for patients abiding by certain religious doctrines. Drawbacks of GIFT are that there is no diagnostic test to determine whether fertilization has occurred and there is an increased chance of having an ectopic pregnancy. Although GIFT is generally more invasive than traditional IVF; it constitutes one of many choices in pursuing assisted reproductive technology.

10.2.4 Zygote Intra Fallopian Transfer (ZIFT)

ZIFT is a combination of IVF and GIFT. The sperm and ova are extracted with the same procedures as IVF and GIFT, and the ova are fertilized outside of the body as with IVF. ICSI may also be used in conjunction with ZIFT, (Refer Figure 10.6). During a ZIFT procedure, the developing embryo is placed in the fallopian tubes at the zygote stage (in contrast to IVF where the developing embryo is placed in the uterus later in its development, at the blastocyst stage). Since the developing embryo is placed in the woman's body much sooner with ZIFT, it is also considered more "natural" than IVF.

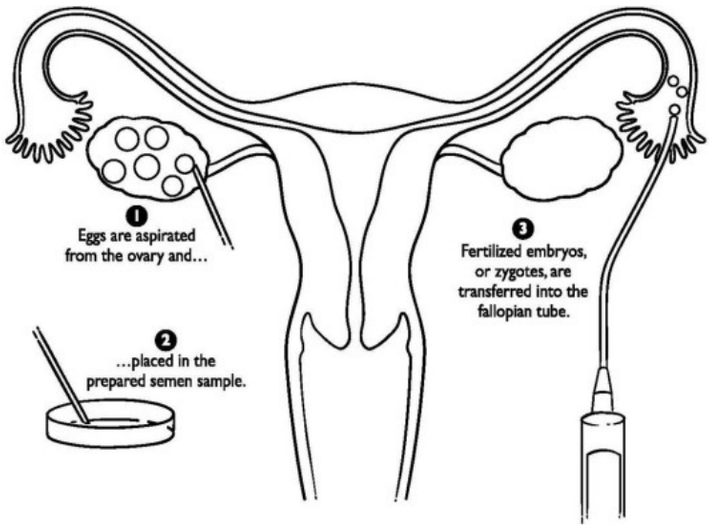

Fig. 10.6 *Showing Zygote Intra Fallopian Transfer (ZIFT)*

Zygote Intra Fallopian Transfer (ZIFT) is an Assisted Reproductive Technology (ART) first used in 1986 to help those who are infertile to conceive a child. ZIFT is a hybrid technique derived from a combination of In-Vitro Fertilization (IVF) and Gamete Intra Fallopian Transfer (GIFT) procedures. Despite

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a relatively high success rate close to that of IVF, it is not as common as its parent procedures due to its costs and more invasive techniques. Some patients prefer ZIFT, however, considering it more natural because the fertilized oocyte, the zygote, is placed in the woman's body for implantation much sooner than with IVF. To be a suitable candidate for ZIFT, a woman must have at least one healthy fallopian tube where the physician can implant the zygote. The entire ZIFT process takes approximately four weeks, including the period when the patient must first undergo hormone treatment called superovulation. With superovulation, the physician administers fertility medications such as Clomid to stimulate the ovaries to produce several mature eggs, or ova. Clomid will increase the amount of Foolicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) in the female that are required for oocyte maturation. If Clomid is not enough to stimulate oocyte maturation, the physician can also inject the patient with additional FSH and LH intravenously to supplement the oral Clomid medication for a more aggressive hormone therapy.

Once the hormone treatment helps produce several mature ova, the physician extracts the ova through a non invasive procedure called transvaginal oocyte retreival, the same technique used with IVF and GIFT. For transvaginal oocyte retreival, a thin needle guided by sonogram is inserted through the vaginal wall and enters the ovaries to extract several mature ova. Then shortly before implantation the physician obtains sperm from the male either by masturbation, by using a collection condom or with surgical methods if there is an obstruction preventing the normal ejaculation of sperm.

Once the sperm and ooctyes are prepared, the physician allows the sperm to fertilize the oocyte in a petridish either naturally or manually with a procedure called Intra Cytoplasmic Sperm Injection (ICSI). If there is a male fertility problem such as low sperm count, a high concentration of misshapen sperm, or low sperm motility; ICSI is a good procedure to be used in conjunction with ZIFT. With ICSI, the sperm is injected directly into the egg in the petri dish to increase the chances of fertlization. Studies are conflicted on whether ICSI will increase the chances of birth defects in newborns, but any increased chance is too low to determine accurately.

After fertilization, in vitro the physician monitors the fertilized oocyte (s) for approximately twenty-four hours until cell division begins. With ZIFT, the physician then implants the zygote into the fallopian tube. This differs from IVF, where the physician waits until the fertilized egg has divided into eight cells before implanting it into the uterus. The location where the physician implants the developing embryo depends on the stage of the embryo's development and thus models the path that the developing embryo would follow after natural conception.

During ZIFT, the physician places one to four zygotes in the fallopian tubes through a surgical technique called laparoscopy, an invasive procedure utilizing a small abdominal incision unlike IVF, where the physician places the 8-cell embryo in the uterus by entering through the cervix. The zygote then travels down the fallopian tube and may implant on the uterine wall. In a healthy young woman,

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there is approximately a 32–36% chance that the fertilized egg will implant in the uterine wall and result in pregnancy. The number of zygotes the physician places in the fallopian tube will depend on the patient's age as well as her preference. The greater the woman's age, the more difficult it becomes for pregnancy to occur, thus physicians may insert more zygotes to increase the chance of a successful implantation and resulting live birth.

As with GIFT, there is a greater chance of an ectopic pregnancy (the fertilized egg implants anywhere other than inside the uterus) when using ZIFT. Although the probability of pregnancy with ZIFT is close to that of IVF, it makes up only 1% or less of all ART currently used in the United States. The invasive surgery of laparoscopy and the relatively higher costs have made ZIFT less appealing than IVF, which due to its popularity has attracted more research and resulted in higher success rates for both fertilizations and live births. ZIFT, like GIFT, followed the development of IVF as another technique to help achieve pregnancy. Currently, every year in the United States approximately 250–280 babies are born as a result of ZIFT compared to 40,000 babies born from all assisted reproductive technologies.

Advantages of ZIFT

Following are the advantages of ZIFT:

- Confirmation of fertilization and selection of only normally fertilized zygotes for transfer.
- Embryo cleavage and development occur in the natural and physiological environment of the fallopian tube
- Better synchronization between embryonic and endometrial development
- Avoidance of suboptimal in vitro culture systems
- Prevention of zona hardening, especially in couples with advanced female partner age
- Prevention of microtrauma to the endometrium by uterine transfer catheters
- Prevention of embryo expulsion following UET induced by Sub endometrial myometrial contractions
- Prevention of the detrimental effects of cervical microorganisms associated with UET
- Important diagnostic information provided by laparoscopy

Disadvantages of ZIFT

Following are the disadvantages of ZIFT:

- Risks and complications inherent with laparoscopy
- Increased cost compared with uterine embryo transfer

- Longer hospital stay compared with uterine embryo transfer
- Lack of the ability to select the morphologically best-cleaving embryos compared with uterine embryo or blastocyst transfer.

ZIFT normally requires general anesthesia and endotracheal intubation. Intrafallopian transfer with local anesthesia and continuous sedation has also been described. ZIFT is performed 18–48 hours after oocyte aspiration using a three-puncture video laparoscopic Technique. During the ZIFT procedure, pronuclear embryos are normally selected for transfer based only on the visualization of two pronuclei 18–24 hours after egg retrieval and insemination.

10.2.5 Intra Cytoplasmic Sperm Injection (ICSI)

One kind of assisted reproductive technology is ICSI that is helping numerous individuals to get rid off from male infertility. Spermatozoa sometimes fail to fertilize even when they are artificially placed in close proximity to eggs during conventional In-Vitro Fertilization (IVF). Fertilization failure in IVF is particularly common where there are grossly abnormal semen parameters or when the number of spermatozoa is insufficient. The ability of ICSI to achieve higher fertilization and pregnancy rates regardless of sperm characteristics makes it the most powerful micro manipulation procedures to treat male factor infertility. In fact, the therapeutic possibilities of ICSI go from cases in which, after sperm selection, the spermatozoa show poor progressive motility, to its application to azoospermicmen where spermatozoa are microsurgically retrieved from the epididymis and the testis.

Injection of single mature immobilized normal spermatozoa into the cytoplasm of a mature metaphase II oocyte is known as Intra Cystoplasmic Sperm Injection (ICSI). Since the introduction of ICSI, it has revolutionized the treatment of male factor infertility and excellent pregnancy and implantation rates are achieved in couples for whom there were no treatment option except donation or adoption. ICSI was first used successfully in patients whose oocytes failed to become fertilized after insemination with motile spermatozoa, (Refer Figure 10.7). Then, it became evident that ICSI might equally be well applied in couples with too few spermatozoa for conventional IVF. Finally researchers tried for azoospermic men by injecting sperm, which obtained from epididymis (Obstructive Azoospermia, OA) and testes (Non Obstructive Azoospermia, NOA). It was also successful in terms of normal fertilization, embryo development and implantation rates as well as birth of healthy offspring 10-12. Before 1992, the majority of severe male factor infertility was virtually untreatable. Due to establishment of ICSI as a routine it is now possible to treat the whole spectrum of male infertility from such optimal ejaculate samples or ejaculatory failure to obstructive and non-obstructive azoospermia.

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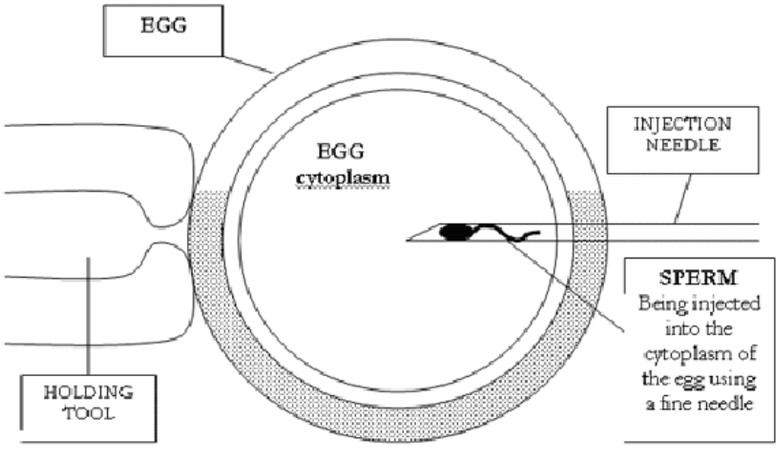

Fig. 10.7 *Showing Intra Cytoplasmic Sperm Injection (ICSI)*

Significance of ICSI

It is used in case of:

- Couples who have suffered from recurrent failure of fertilization due to disorder at functional level of gametes, barrier at the level of acrosome reaction, zona binding or interaction, zona penetration or fusion with oolema. In ICSI all these steps are bypassed and only requirement is the decondensation of spermatozoa inside the oocyte. Severe oligospermia where sperm count is less than 5million/ml., severe oligospermia and testicular failure. Oligopsermia due to hypogonadotropic hypogonadism, environmental factors, drugs or due to any disease can be corrected by behavioral changes and specific treatment. Otherwise repeated low sperm count with high FSH and without any specific reason (idiopathic) or Y chromosomal micro deletion are the candidates for ICSI. Severe asthenospermia including patients with ultra-structural abnormalities such as kartagener's syndrome.
- Teratospermia where >70% sperms are morphologically abnormal.
- Obstructive azoospermia due to congenital absence of Vas deference, vasectomy or post inflammatory obstruction of the vas deference. Sperm can be retrieved by Per Epididymal Sperm Aspiration (PESA), Testicular Sperm Aspiration (TESA) or Testicular Sperm Extraction (TESE).
- Non-Obstructive Azoospermia. Sperm can be retrieved by TESA, TESE or open biopsy of the testis.
- Ejaculatory dysfunction such as retrograde ejaculation.
- Paraplegeic male if electro ejaculation is not satisfactory, then TESE and ICSI can be done.
- Immunological factors-Anti-sperm antibody in both male and female partner.

- Frozen semen sample in patients having chemotherapy and radiotherapy. Testicular biopsy specimen may also be cryopreserved as backup where quality of ejaculation is inadequate for freezing.

Thus ICSI is one of the most successful technique to overcome infertility as it involves injection of mechanically immobilized spermatozoa achieves fertilization at a higher rate than the injection of motile spermatozoa directly inside the woman

10.2.6 Assisted Hatching

Assisted hatching is a laboratory procedure that is sometimes done along with In-Vitro Fertilization (IVF) treatment. IVF involves mixing eggs with sperm in a laboratory (as opposed to within a woman's body like in natural conception), (Refer Figure 10.8). Eggs are considered fertilized when a sperm succeeds in penetrating the egg. During IVF, the fertilized eggs are monitored for 3 to 6 days as they divide and develop into embryos. The best embryo can then be placed into the woman's uterus (embryo transfer) in the hopes of helping her become pregnant or it can be frozen for future use. While the embryo develops, it is surrounded by cells that make up a protective shell (Zona Pellucida). The embryo naturally breaks out of this shell as it grows. Occasionally, the doctor may ask the laboratory to make a small 'crack' in the outer shell of the embryo right before it is placed into the woman's body (assisted hatching) with the hope is that assisted hatching might help the embryo expand, implant into the uterine wall, and finally lead to a pregnancy. During assisted hatching, the outer shell of the embryo is artificially weakened by making a small hole in the Zona Pellucida. This can be done in several different ways. One method involves the application of an acid solution, called Tyrode's solution, to help melt a small hole in the shell. Another method involves the use of a laser to 'crack' the shell.

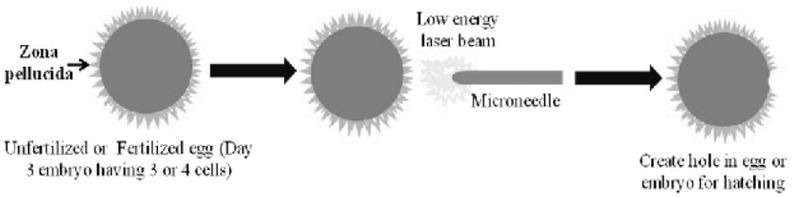

Fig. 10.8 *Laser Assisted Hatching Procedure*

Rarely, assisted hatching can damage the embryo, making it unusable. The risk for identical twins might be slightly increased when assisted hatching is applied. Medical complications are higher in identical twin pregnancies than in normal, singlet on pregnancies. Medicines such as antibiotics and steroid hormones are sometimes prescribed around the day of the assisted hatching and embryo transfer. Uncommonly, side effects can occur from the use of these medications.

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10.3 MONITORING OF OVULATION PHASE

The ultrasound technique provides more accurate information of follicle number and size than can be obtained by serum estrogen determinations alone. Under optimal conditions a follicle in the ovary can be visualized from a diameter of 2–3 mm. The follicles appear as echo-free structures amidst the more echogenic ovarian tissue. Measurement of the internal diameter of the follicle in two planes and the average diameter is done. Follicles usually grow by 2–3 mm per day. Complete elimination of the use of paper charts in recording follicular progress instead of electronic graphs are used which allow the determination of follicular sizes, endometrial thickness and serum estradiol levels. All computers are connected to the network, allowing access from any terminal in the center. Following the ultrasound scan, the patient has a consultation with her treating physician with help of the computer in revealing all the characterstics of the cycle, including the number of follicles, dynamics of follicular growth, endometrial thickness and changes in the type of ovulation regimen are visualized as well as discussed.

Therefore, ultrasound monitoring of follicular growth is the most important tool in the assessment of progress in ovarian stimulation. With follicles less than 24 mm in size, with increasing size the likelihood of obtaining mature oocyte increases. However, there is no difference in the oocyte quality obtained from follicles between 18 and 22 mm in diameter. This allows more convenient and predictable planning of oocyte collection. Quantitative and qualitative assessment of perifollicular flow, allow for a more accurate assessment of follicular competence. Follicles that have >75% of their surface perfused, or where PSV is >10 cm/s, appear to contain an oocyte of satisfactory quality.

Check Your Progress

1. What is a step towards maximizing your chance of conceiving naturally?
2. What is superovulation?
3. What is cryopreservation?
4. What is intracellular ice formation?

10.4 SUPEROVULATION

Super-ovulation is a critical requirement for successful assisted reproduction technology in horses. Management techniques to optimize fertility in the horse have been limited by mares ovulating only one follicle per cycle refer, figure 10.9. Induction of multiple ovulations in the mare would increase the number of oocytes available for fertilization. Consequently, increasing the ovulation rate of sub fertile mares or of normal mares bred to subfertile stallions may increase the probability

of establishing a pregnancy. Superovulation also may enhance embryo collection rates from donor mares, increasing the efficiency of embryo transfer programs. Finally, stimulation of multiple large follicles may increase the collection efficiency of oocytes which can be used for In-Vitro Fertilization (IVF), Gamete Intra Fallopian Transfer (GIFT) and Intra Cytoplasmic Sperm Injection (ICSI). Super ovulation also known as Controlled Ovarian Hyperstimulation, is the process of inducing a woman to release more than one egg in a month. It is different from ovulation induction where the goal is to release one egg per month.

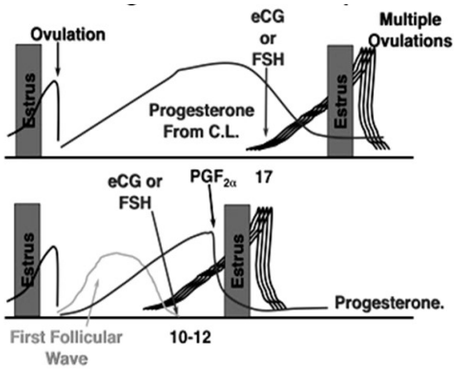

Fig. 10.9 *Stimulation of Follicular Development for Super-Ovulation*

Needs of Superovulation

Woman with open fallopian tubes and whose partner has adequate sperm count are candidates for super ovulation. If a woman already ovulates and is not conceiving, doctors can increase her chances of getting pregnant by causing her to release more eggs by super ovulation. Similarly when a woman has been ovulating with an oral medication such as Clomiphene and is still not conceiving by stimulating her ovaries to release more egg.

Procedure of Supperovulation

Woman who naturally ovulates may release extra eggs when they take oral medications such as clomiphene. This is a mild super ovulation and is generally less expensive and is low risk bearing. Initially an ultrasound is performed around the time of ovulation to determine how many follicles are growing if the result of ultrasound reveals that the woman is producing just one follicle the dose or medication might be changed in the next cycle. Sometime Gonadotopins are also used for super ovulation. Gonadotropins are those hormones that cause eggs to grow. Gonadotropins are injected inside the woman (or in form of liquid) e.g., Bravelle, Follistin, Gonal-F, etc. One main disadvantage of administration of gonadotropins is the growth of multipe eggs at one time. To prevent this situation, close and regular monitoring of ovaries is required. Once the desired number of eggs gets mature, the woman is given Human Choroinic Gonadotropin injection to cause ovulation.

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Risks of Superovulation

Following are the risks of superovulation:

- **Multiple Births:** The most common form of human multiple birth is twins (two babies), but cases of triplets (three), quadruplets (four), quintuplets (five), sextuplets (six), septuplets (seven), and octuplets (eight) have all been recorded with all siblings being born alive. Multiple births can occur due to many reasons. It has occurred naturally too but of late the fertility treatments seem to have caused a few more multiple births. Multiple births of more than 6 babies are heard from time to time and many times it is because of one of the fertility treatments. The fertility treatments lead to a spurt of egg production in a woman during her ovulation cycle. As it is too risky to fertilize and place only one embryo in the uterus, it is the usual practice in fertility treatment to place 5, 6 or more embryos. Now, if all or more than one embryo develops, it becomes multiple births.
- **Adnexal Tortion (Ovarian Twisting):** Rare complication that occurs in one percent of cycles. As the ovaries gets enlarge, they may twist, cutting off their blood supply and ceasing severe abdominal pain, nausea, vomiting and sometimes low grade fevers. It can be treated by surgical untwisting of ovaries.
- **Ectopic Pregnancy:** An ectopic pregnancy occurs when a fertilized egg implants itself outside the uterus. Egg may implant in the fallopian tube or less commonly in the ovary, cervix or pelvic cavity. This condition occurs in 1-2% of all pregnancies. Most of the infertility treatments results in the ectopic pregnancies as many woman with infertlity dysfunction and treatment leads to release of multiple eggs thereby increasing the possibility that not all fertilized eggs move through the tubes into the uterus. Ectopic pregnancies require emergency medical treatment and the pregnancy must be ended soon as the life of both mother and fetus is on stake.

10.5 CRYOPRESERVATION

Cryopreservation is an applied aspect of cryobiology which permits low temperature for the maintenance of diversity of the cells. However freezing process is fatal to most living organisms, since both intra- and extracellular ice crystals are formed and results in changes to the chemical setting of cells that lead to cellular mechanical constraints and injury, (Refer Figure 10.10). The major hurdle for cells to overcome at low temperatures is the water-to-ice phase transition. Cell injury at fast cooling rates is attributed to intracellular ice formation, whereas slow cooling causes osmotic changes due to the effects of exposure to highly concentrated intra- and extracellular solutions or to mechanical interactions between cells and the extracellular ice. The successful cryopreservation of cells and tissues has been gradually increasing in recent years, with the use of cryoprotective agents

and temperature control equipment. Main objective of cryopreservation is to reduce the damage of biological material which encompasses various cells and tissues such as bacterial, fungal, plant and mammalian cells at low temperature for the process of freezing and storage. Technique of cryopreservation provides a source of preserving tissues and living cells for research and biomedical purposes.

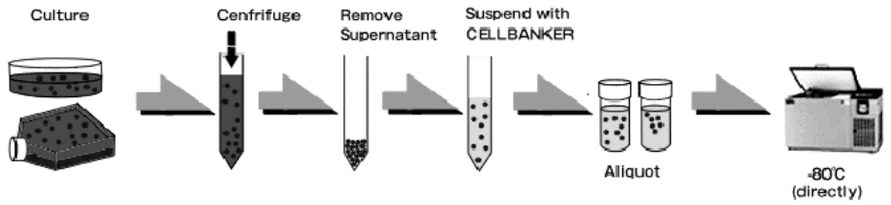

Fig. 10.10 *Schematic Presentation of Procedure of Cryopreservation of a Culture*

Cryoprotectants

In the process of cryopreservation, biological samples are usually maintains in a state of suspended animation at cryogenic temperature for any considerable period and is used to preserve the fine structure of cells. The freezing behavior of the cells can be altered in the presence of a Cryo Protective Agent (CPA or Cryoprotectant), which affects the rates of water transport, nucleation and ice crystal growth. Different cryoprotectants which are widely used in the process of cryopreservation are as follow:

- **DMSO (Dimethysulfoxide):** It was first synthesized by Alexander Zaytsex in 1866 and is commonly used for the cryopreservation of mammalian cells because of low toxicity levels. However use of DMSO is disadvantageous at certain point of time as it reduces the survival rate and the induction of cell differentiation which is caused by DNA methylation and histone alteration. So these negative effects of DMSO in cryopreservation create hindrances for its use in routine clinical applications.
- **Glycerol:** Glycerol is discovered as CPAs in 1949 and it is one of the most commonly used cryoprotectant because of its effectiveness. It is a non electrolyte compound widely used for the cryopreservation of the bacterial cells and the spermatozoa. Both of these cryoprotectants act by reducing the electrolyte concentration in the residual unfrozen solution within in the cell at any given temperature.
- **Proteins:** In the process of cryopreservation proteins are also used as cryoprotectants and one such example is 'Sericin' which is a water soluble sticky protein, having a molecular weight of nearly 30KDa and isolated from the silkworm cocoon. It is a suited cryopreservative for the progenitor cells or the hepatocytes.
- **Cell Banker Series:** For rapid cell cryopreservation at "80°C and better survival rates of freezing and thawing, new different types of cryopreservation

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agent has been developed called as Cell Banker Series by Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan. Cell Banker Series contains 10% DMSO, glucose and prescribed high polymer and pH adjustors. Serum containing cell banker series used for the cryopreservation of the mammalian cells, whereas Cell Banker 3 or Stem Cell Banker contains 10% DMSO and other inorganic compound are suitable for the preservation of somatic cells and induced pluripotent stem cells.

Procedure of Cryopreservation

The process of cryopreservation and its procedure varies with different types of cells lines. Generally the process of cryopreservation involves following steps: i. Slow freezing, ii. Vitrification, iii. Subzero non-freezing storage, and iv. Preservation in dry state. Generally, the storage of mammalian cells in the dry state is not readily possible because of difficulties in introducing the disaccharide trehalose (disaccharide of glucose, 342Da) and amino acids (used as preservatives in plants) into the intracellular region. The major steps in cryopreservation are:

- The mixing of CPAs with cells or tissues before cooling
- Cooling of the cells or tissues to a low temperature and its storage
- Warming of the cells or tissues
- Removal of CPAs from the cells or tissues after thawing

The appropriate use of CPAs is therefore important to improve the viability of the sample that to be cryopreserved.

Cryopreservation can be achieved by two methods either by Slow Freezing or either by Vitrification. Major distinction between these two methods is the concentration of CPAs and the cooling rates used during cryopreservation. Theoretically, if the cooling rate is low than cells could efflux the intracellular water, there is enough time to eliminate the super cooling which proves advantageous to prevent intracellular ice formation. That's why there are differences present in the capacity of different cells to move water across plasma membrane and thus cooling rates varies with different cell types.

- **Slow Freezing:** It encompasses the water within the cytoplasm with CPAs which reduces the cell damage and adjusts the cooling rate in accordance to the permeability of cell membrane. Cooling rate for this is about 1°C/min in the presence of CPA whose concentration is less than 1.0 M along with the use of Bench top portable freezing container. The biggest advantage of slow freezing is the reduction in the rate of the Contamination throughout the procedure. However the disadvantage of slow freezing is that it has high risk of the freeze injury due to the formation of extracellular ice.
- **Vitrification:** It is the process that involves the direct transformation of cell suspension from the aqueous phase to a glass state after the direct exposure to the liquid nitrogen. Simultaneously, the process requires the cooling of

cells or tissues to a deep cryogenic temperature after treating with the CPAs with subsequent cooling to prevent ice nucleation. Process of vitrification is largely depends upon 3 factors: i. Viscosity of the sample, ii. Cooling and warming rates, and iii. Sample balance. Therefore the balance should be maintained between all the above mentioned factors to ensure the success of vitrification. Further the vitrification has 2 methods: Equilibrium vitrification and Non equilibrium vitrification.

Equilibrium vitrification requires the formulation of multimolar cryoprotectant mixtures and their injection into the cell suspension. Whereas the **non equilibrium vitrification** which is further divided into carrier-based system includes the former plastic straws, quartz micro capillaries and cryoloops for obtaining a minimum drop volume and carrier-free systems involves the use of an extremely high freezing rate along with lower concentrations of the CPA mixture. Major advantage of vitrification is the low risk of freeze injury, thereby ensuring a sufficiently high cell survival rate. However, the high potential of contamination with pathogenic agents is present and therefore the technique requires good manipulation skills.

Advantages of Cryopreservation

Following are the advantages of cryopreservation:

- Possible banking of cells for human leukocyte antigen typing for organ transplantation
- The allowance of sufficient time for transport of cells and tissues among different medical centers
- The provision of research sources for identifying unknown transmissible diseases or pathogens.
- The long-term storage of stem cells is highly beneficial in tissue engineering, which leads to regeneration of soft tissue esthetic function and for the treatment of known diseases having no current cure.

Applications of Cryopreservation

Cryopreservation has numerous advantages in following areas such as:

- Cryopreservation of cells or organs
- Cryosurgery
- Biochemistry and molecular biology
- Food sciences
- Ecology and plant physiology
- Medical applications such as blood transfusion, bone marrow transplantation, artificial insemination and *in vitro* fertilization.

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Examples of Cryopreservation

Following are the few examples of cryopreservation:

- **Oocytes and Embryo:** Cryopreservation of the embryo was done for the first time in 1996, with the application in IVF cycle prior to chemotherapy in a woman diagnosed with breast cancer. Cryopreservation of mature oocytes is a proven technique for preserving the reproductive capacity of the infertile or diseased women.
- **Sperm, Semen and Testicular Tissue:** Cryopreservation is used as a first-line means for preserving fertility in men who have undergone vasectomy or treatments that may compromise their fertility, such as chemotherapy, radiotherapy, or surgery.
- **Hepatocytes:** Isolated hepatocytes have wide range of applications in science and medicine over the past 40 years in areas such as physiological studies, investigations on liver metabolism, organ preservation &drug detoxification and experimental &clinical transplantation. In addition to this current interest is increasing in the applications of liver progenitor cells across a range of scientific areas, including both regenerative medicine and biotechnology, which raises the need for cryobanking.
- **Islets:** Development of islet cryopreservation methods has been ongoing, but results are still suboptimal, with a survival rate of less than the cryopreservation of the primary neuronal cells and cardiomyocytes as they are routinely used in the neuroscience and cardiology research.

Limitations of Cryopreservation

Despite of the numerous advantages of the cryopreservation technique both in basic and clinical research, there are some limitations also. Cells that metabolize at low temperatures such as "196°C (i.e., in liquid nitrogen), have inevitable side effects, including a genetic drift toward biological variations of cell-associated changes in lipids and proteins that could result in the impairment of cellular activity and structure. If there were no limit to the amount of CPA that could be used, cells would be preserved perfectly. In conventional settings, however, CPAs themselves can be damaging to cells, especially when used in high concentrations. For example, there is a possibility that DMSO may alter chromosome stability, which can lead to a risk of tumor formation. Apart from endogenous changes in cells, the possible infection or contamination with cells such as tumorous ones should be prevented.

Check Your Progress

5. How are the sperm and ova extracted?
6. What is super-ovulation?
7. What is super ovulation also known as?
8. What does cryopreservation permit?
9. How can cryopreservation be achieved?

10.6 ANSWERS TO CHECK YOUR PROGRESS

NOTES

1. Ovulation cycle tracking is a good first step towards maximizing your chance of conceiving naturally.
2. Superovulation is a term used to describe the drug-induced production of multiple eggs for use in assisted reproductive technologies such as in IVF.
3. Cryopreservation is the use of very low temperatures to preserve structurally intact living cells and tissues.
4. Intracellular ice formation in particular is a critical issue that has to be controlled to keep the cell membrane intact and the cells alive.
5. The sperm and ova are extracted with the same procedures as IVF and GIFT, and the ova are fertilized outside of the body as with IVF.
6. Super-ovulation is a critical requirement for successful assisted reproduction technology in horses.
7. Super ovulation also known as Controlled Ovarian Hyperstimulation,
8. Cryopreservation is an applied aspect of cryobiology which permits low temperature for the maintenance of diversity of the cells.
9. Cryopreservation can be achieved by two methods either by Slow Freezing or either by Vitrification.

10.7 SUMMARY

- Infertility is the central issue in the lives of individuals who suffers from it. Interest of people in Assisted Reproductive Technology (ART) has remained high since the birth of the world's first in vitro fertilization baby, Louise Brown, in the United Kingdom.
- Assisted Reproductive Technology (ART) is used to treat infertility. It includes fertility treatments that handle both a woman's egg and a man's sperm. It works by removing eggs from a woman's body.
- Superovulation is a term used to describe the drug-induced production of multiple eggs for use in assisted reproductive technologies such as in IVF.
- Cryopreservation is the use of very low temperatures to preserve structurally intact living cells and tissues.
- Intracellular ice formation in particular is a critical issue that has to be controlled to keep the cell membrane intact and the cells alive.
- Infertility is the central issue in the lives of individuals who suffers from it. It is a source of social and psychological suffering for both men and women within the couple.

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- Fertility drugs alone, such as clomiphene citrate, are usually tried first if anovulation (the lack of ovulation) seems to be the only cause of infertility.
- Intra Uterine Insemination (IUI) is considered one of the oldest techniques to treat infertility and dates back to the early 1900s.
- During IUI, the seminal fluid is prepared in the lab (washed with special media), injected inside the uterus after stimulating the ovaries to produce more eggs per cycle.
- The sperm used in the procedure is obtained either by masturbation, by a collection condom, or surgically through an incision in the testes if a blockage is preventing the normal ejaculation of sperm.
- Since often more than one egg is placed in the uterus, the likelihood of more than one implanting is also relatively high, bringing the possibility of multiple births.
- GIFT is a procedure initially developed by Dr. Ricardo Asch in the 1980s. In this technique the physician extracts the ova and sperm with the same procedures as in IVF, but the ova are not fertilized in vitro (outside of the body).
- Gamete Intra Fallopian Transfer (GIFT) has emerged as one of the major Assisted Reproductive Technologies (ART).
- GIFT was developed out of a desire to place gametes directly into their natural physiologic environment in order to enhance the potential for fertilization.
- The GIFT process begins by obtaining the father's semen two hours before the mother undergoes a laparoscopic procedure to harvest her eggs.
- The sperm and ova are extracted with the same procedures as IVF and GIFT, and the ova are fertilized outside of the body as with IVF.
- As with GIFT, there is a greater chance of an ectopic pregnancy (the fertilized egg implants anywhere other than inside the uterus) when using ZIFT.
- Spermatozoa sometimes fail to fertilize even when they are artificially placed in close proximity to eggs during conventional in vitro fertilization (IVF).
- Assisted hatching is a laboratory procedure that is sometimes done along with In-Vitro Fertilization (IVF) treatment.
- The ultrasound technique provides more accurate information of follicle number and size than can be obtained by serum estrogen determinations alone.
- Ultrasound monitoring of follicular growth is the most important tool in the assessment of progress in ovarian stimulation.
- Superovulation is a critical requirement for successful assisted reproduction technology in horses.

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- Super ovulation also known as Controlled Ovarian Hyperstimulation, is the process of inducing a woman to release more than one egg in a month.
- The ultrasound technique provides more accurate information of follicle number and size than can be obtained by serum estrogen determinations alone. Under optimal conditions a follicle in the ovary can be visualized from a diameter of 2–3mm.
- Cryopreservation is an applied aspect of cryobiology which permits low temperature for the maintenance of diversity of the cells.
- Freezing process is fatal to most living organisms, since both intra- and extracellular ice crystals are formed and results in changes to the chemical setting of cells that lead to cellular mechanical constraints and injury.
- Technique of cryopreservation provides a source of preserving tissues and living cells for research and biomedical purposes.

10.8 KEY WORDS

- **Assisted hatching:** It is a laboratory procedure that is sometimes done along with In-Vitro Fertilization (IVF) treatment.
- **Superovulation:** Super-ovulation is a critical requirement for successful assisted reproduction technology in horses. Management techniques to optimize fertility in the horse have been limited by mares ovulating only one follicle per cycle.
- **Multiple births:** The most common form of human multiple birth is twins (two babies), but cases of triplets (three), quadruplets (four), quintuplets (five), sextuplets (six), septuplets (seven), and octuplets (eight) have all been recorded with all siblings being born alive.
- **Adnexal tortion (Ovarian twisting):** It is a rare complication that occurs in one percent of cycles. As the ovaries gets enlarge, they may twist, cutting off their blood supply and ceasing severe abdominal pain, nausea, vomiting and sometimes low grade fevers. It can be treated by surgical untwisting of ovaries.
- **Ectopic pregnancy:** It occurs when a fertilized egg implants itself outside the uterus. Egg may implant in the fallopian tube or less commonly in the ovary, cervix or pelvic cavity.
- **Cryopreservation:** It is an applied aspect of cryobiology which permits low temperature for the maintenance of diversity of the cells.

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10.9 SELF ASSESSMENT QUESTIONS AND EXERCISE

Short Answer Questions

1. Name one fertility drug used commonly for Assisted Reproductive Techniques.
2. When was the cryo preservation of embryo done for the first time?
3. Define the process of Vitrification.
4. What is the disadvantage of using Slow freezing Technique during cryo preservation?
5. Expand the terms ZIFT, GIFT, ICSI.

Long Answer Questions

1. Write the following in detail:
   - Assisted hatching
   - Intra Cytoplasmic Sperm Injection ICSI
2. Explain the process of superovualtion.
3. Illustrate deeply the process of cryopreservation.
4. How the infertility ratio gets decreased after birth of first test tube baby?
5. How is the monitoring of superovulation phase done?

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UNIT 11 SPERM AND ARTIFICIAL INSEMINATION

NOTES

Structure

11.0 INTRODUCTION

These days infertility is very common, surprisingly the ratio is not high only in women but in men also. Infertility affects an estimated 15% of couples globally, amounting to 48.5 million couples. Males are found to be solely responsible for 20-30% of infertility cases and contribute to 50% of cases overall. Male factor infertility is the primary medical issue in about 30% of all infertility cases. There are many causes of male infertility such as low sperm production, abnormal sperm function or blockages that prevent the delivery of sperm. Illnesses, injuries, chronic health problems, lifestyle choices, stress etc. To overcome the problem of infertility sperm banking is one of the option.

Sperm banking or sperm bank also referred as cryobank is a facility that that collect, freezes, and stores human sperm. It is a highly effective method of protecting male fertility potential. Cryopreservation of semen is a vital procedure which can be employed for a variety of purposes, including donor insemination and the preservation of gametes in patients undergoing gonadotoxic treatment. It also may be helpful to fertile couples who experience difficulty conceiving. The purpose of cryopreserving semen (sperm banking) is to help ensure the possibility of conception in the future.

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In this unit, you will study about sperm banking, Artificial Insemination, IVF, embryo transfer and test tube babies, Gene Knock-Out and Knock-In.

11.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand the sperm banking and artificial insemination
- Explain the, IVF, embryo transfer and test tube baby
- Discuss about gene knock in and knock out

11.2 SPERM BANKING

Gamete cryopreservation is the technical name for sperm banking and is the cooling and storage of sperm at very low temperatures for a prolonged period. Men may choose to bank sperm if there is a possibility of losing fertility. This is an important option for men who have not established a family or whose family is not yet complete. All men who hope to father a child in the future should consider cryopreservation of semen or testicular tissue, even if the specimens have low numbers of sperm. Semen for cryopreservation is obtained by masturbation, and must be brought to the ART (Assisted Reproductive Technology) laboratory within 1 hour of being produced. Once in the laboratory, an analysis of the quantity and quality of sperm is made and a small test vial is prepared. The remaining sample is divided into smaller amounts and transferred into multiple vials. The number of vials stored depends on the total volume of the sample, and the number of mobile sperm in each milliliter. The entire freezing process is completed in about 3 hours. The following day, the test vial is thawed, and an examination of the number and motility of the defrosted sperm is performed. As infertility rates increase, more and more couples are using Assisted Reproductive Technologies (ART), including artificial insemination and IVF, to reach their dreams of parenthood. The increased demand for these procedures has resulted in an increase in sperm banking. A sperm bank, also referred to as a cyrobank, is a facility that collects, freezes, and stores human sperm. The sperm kept at a sperm bank is either donated by men to be used by couples seeking sperm donations for artificial insemination or IVF procedures, or is provided by men who want to preserve their own sperm for future use,

Artificial Insemination is a fertility treatment method used to deliver sperm directly to the cervix or uterus in the hopes of getting pregnant. Sometimes, these sperm are washed or 'prepared' to increase the likelihood a woman will get pregnant. Two chief approaches to artificial insemination exist: IntraUterine Insemination (IUI) and Intra Cervical Insemination (ICI). Some women may also take medications to stimulate ovarian follicle growth and increase conception chances. Conceiving requires a man's sperm to travel up the vagina, through the

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cervix, into the uterus, and into a fallopian tube where an egg is fertilized. However, sometimes a man's sperm isn't mobile enough to make this trip. Other times, a woman's cervix may not be favorable to allow sperm to travel into the uterus. In these instances and other situations, artificial insemination may help a woman conceive. Artificial Insemination (AI) consists of placing sperm, previously selected from a sample, in the woman's uterus. The sperm can be provided either by the partner (AIH) or from a sperm bank (AID). In order to increase the chances of pregnancy the ovaries are stimulated with hormones and ovulation is monitored in order to know the best time to carry out the insemination procedure. Sperm can either be provided by the partner (Artificial Insemination with Husband sperm, AIH) or from a sperm bank (Artificial Insemination with Donor sperm, AID). The semen is prepared in the laboratory, where mobile sperm are separated from the other components (seminal plasma and other cells). To increase the chances of a pregnancy, the ovaries are stimulated with hormones and ovulation is monitored to ascertain the best time to carry out the insemination procedure.

There are many couples out there who find it difficult to conceive a baby. Well, there are several reasons that contribute to this ever increasing problem. Poor eating habits, stress and lifestyle are some of the main factors that contribute to this problem. Test tube baby refers to a term used for a child that is conceived outside the body of a woman by a scientific procedure known as in vitro fertilization, also known as IVF Treatment. This entire procedure is done in a laboratory. In this process of IVF, eggs from the ovary of a mother are taken and fertilized by the father's sperm. Then the fertilized eggs are cultured for two to six days and allowed to be divided two to four times inside a test tube, thus the name test tube baby. These eggs are then implanted back into the uterus of a mother where it can develop normally. This is done with the sole intention for establishing and ensuring a successful pregnancy. A test-tube baby is the product of a successful human reproduction that results from methods beyond sexual intercourse between a man and a woman and instead utilizes medical intervention that manipulates both the egg and sperm cells for successful fertilization. The term was originally used to refer to the babies born from the earliest applications of AI and has now been expanded to refer to children born through the use of IVF, the practice of fertilizing an egg outside of a woman's body. The use of the term in both media and scientific publications in the twentieth century has been accompanied by discussion as well as controversy regarding the ethics of reproduction technologies such as AI and IVF. The evolution of these terms over time mirrors the perception of our ability to manipulate the human embryo, as seen by the general public as well as the scientific community. The term 'test-tube baby', prior to the development of IVF technologies in the twentieth century, was used to refer to babies born as a result of AI. W. Pancoast, a physician from Philadelphia, performed the first AI that led to a successful birth in 1884, marking the birth of the first test-tube baby. Despite the fact that this was the earliest instance of any sort of physician-assisted reproduction, the grandeur of the event was not recognized by the public or media in any notable way.

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In molecular cloning and biology, a knock-in (or Gene Knock-In) refers to a genetic engineering method that involves the one-for-one substitution of DNA sequence information in a genetic locus or the insertion of sequence information not found within the locus. Typically, this is done in mice since the technology for this process is more refined and there is a high degree of shared sequence complexity between mice and humans. The difference between knock-in technology and traditional transgenic techniques is that a knock-in involves a gene inserted into a specific locus, and is thus a 'targeted' insertion. A common use of knock-in technology is for the creation of disease models. It is a technique by which scientific investigators may study the function of the regulatory machinery (for example, promoters) that governs the expression of the natural gene being replaced. This is accomplished by observing the new phenotype of the organism in question. The BACs (Bacterial Artificial Chromosomes) and YACs (Yeast Artificial Chromosomes) are used in this case so that large fragments can be transferred.

A Gene Knockout (KO) is a genetic technique in which one of an organism's gene is made inoperative ('knocked out' of the organism). However, KO can also refer to the gene that is knocked out or the organism that carries the gene knockout. Knockout organisms or simply knockouts are used to study gene function, usually by investigating the effect of gene loss. Researchers draw inferences from the difference between the knockout organism and normal individuals. The KO technique is essentially the opposite of a gene knock-in. Knocking out two genes simultaneously in an organism is known as a Double Knock-Out (DKO). Similarly the terms Triple Knock-Out (TKO) and Quadruple Knock-Outs (QKO) are used to describe three or four knocked out genes, respectively. However, one needs to distinguish between heterozygous and homozygous KOs. In the former, only one of two gene copies (alleles) is knocked out, in the latter both are knocked out. Gene knockout is to remove a gene from the cell's DNA so that it is impossible for it to be expressed. There will be absolutely none of the gene product in the cell. Gene knock in is to introduce a new gene to your cell's DNA, which wasn't there before. This means there will now be a new gene product in your cell that wasn't there before. Gene knockdown is to reduce the levels of expression of a gene in your cell. This is not as dramatic as a gene knockout, and there may still be some expression of your gene.

These are molecular genetic techniques which are really useful for studying the function of a gene. For all of these techniques your basic experiment would be:

- Find a gene that you are interested in
- Perform one of the three techniques with your gene
- See what happens to your cell

Historically, it was easier to knock down. A popular technique for this was (and still is) RNA interference. Recently, however, we have got much better at gene knockout using a technique called CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat). Sometimes knocking out a gene completely might kill

your cell, which doesn't tell you much about what your gene does, other than the fact that your cell needs it. By just knocking down the gene, you might be able see how your cell copes at low levels of the gene without killing it.

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11.2.1 Procedure of Sperm Banking

Two steps that are involved in the sperm banking are semen collection and sperm cryopreservation:

- **Semen Collection**: Semen for sperm cryopreservation is generally obtained by masturbation. Prior to the collection of semen, sexual abstinence for at least 2 days, but not more than 5 days, is recommended to maximize the quality of the sample for cryopreservation. For many men, this may be an embarrassing or uncomfortable process. It is critical that men understand how to collect semen and that they be offered a private and relaxing environment to do so. Alternatively, men may collect a semen sample at home or another location than the clinic, providing that they keep the specimen at body temperature and return it to the lab within approximately 45 minutes to 1 hour after collection. Lubricants should be avoided as they can contaminate the specimens. The entire specimen should be collected, particularly in light of the fact that more sperm are present at the beginning of the ejaculate than at the end. Wide-mouth specimen containers should be tested by each laboratory to ensure that they are compatible with semen collection and are not harmful to sperm.
- **Sperm Cryopreservation**: The freezing of spermatozoa was first described in the beginning of the 18th century the quality of the sample for cryopreservation. When semen is cryopreserved, a small aliquot is frozen separately, thawed, and reanalyzed after the initial freeze. This 'test thaw' allows the post thaw survival to be determined as it can vary among individuals and even among different ejaculates from the same person. Post thaw sperm motility is a good representation of the entire ejaculate and gives a reliable estimate of the total motile sperm count for that sample in the future.

For the cryopreserving the sperms, broadly two types of Cryo Preserving Agents (CPAs) are used those are of low molecular weight and protect the sperms from freezing damage or ice crystallization but there is one disadvantage of using cryopreservative agents is that they prove highly toxic if used in high concenteration. One type of CPAs is peneteration CPA and other is non- peneteration CPA. The penetrating agents are small, non-ionic molecules those are lipophilic in nature and are highly miscible with water and allow the sperms to easily peneterate the plasma memberane of the egg, for example, glycol, ethylene, dimethylysulfoxide, glycerol etc. Whereas, the non-peneterating CPAs are high molecular weight molecules those increases the viscosity and lower the freezing point of extra cellular fluid and promotes cellular dehydration. Along with this these agents minimizes the intracellular crystallization by increasing the viscosity of the sample. This is how the sperms are being collected and preserved.

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11.2.2 Types of Sperm Banking or Cryobanking

Cryobanking is of two types:

- **Short-Term Semen Cryobanking:** Short-term semen cryobanking is the depositing, freezing and storage of sperm at a sperm bank for less than one year. Cryobanked sperm is then used in Artificial Insemination, In-Vitro Fertilization (IVF) and other fertility treatment procedures. Short-term semen cryobanking is recommended to preserve semen for deferred inseminations when an intimate partner is temporarily absent. It is also recommended in cases of oligozoospermia (low sperm counts) where multiple semen collections and pooling may be desirable for use in a single insemination. Short-term storage is also performed prior to assisted reproductive technologies (i.e., in vitro fertilization, gamete intrafallopian transfer, etc.) to secure a good quality semen specimen for the prospective procedure.
- **Long-Term Semen Cryopreservation:** Proven, time-tested techniques enable semen specimens and embryos to be frozen and stored indefinitely in liquid nitrogen. According to the donor's specific wishes, these specimens and embryos can later be thawed and used in an attempt to conceive through artificial insemination, IVF or other fertility treatment techniques.

Sperm Banking and Cancer

Preservation of sperms has proved very advantageous for the cancer patients as the chemotherapy negatively affects the process of spermatogenesis, either transiently or permanently. Drugs used in the chemotherapy directly damages proliferating cells, as the early differentiating sperm cells or spermatogonia are extremely sensitive to all these drugs. However, even the relatively quiescent sperm precursors can be damaged due to progressive effects of multiple doses of drugs used in the chemotherapy. In the later stages of spermatogenesis, spermatocytes and spermatids are less sensitive to chemotherapeutic drugs since they are not dividing, and this accounts for the finding of some sperm immediately following chemotherapy with a slow decline in counts over the ensuing months. Leydig cell function appears to be less affected by chemotherapy. Not only the chemotherapeutic drugs affect the process of spermatogenesis but the radiations therapy as well affects the process either transiently or permanently by directly inducing DNA damage and also affects gonadal function.For treating the testicular cancer surgical procedures as Retroperitoneal Lymph Node Dissection (RPLND) are used that is responsible for the ejaculatory dysfunction. That is why sperm banking is very useful for cancer patients.

Significance of Cryopreservation of Sperms

Some men discover they have diseases or must undergo treatment or surgery, which will cause permanent sterilization or genetic damage. As cancer detection and treatment techniques improve, more conditions such as cancer are being

detected at younger ages in men, with longer survival rates. Therefore, more men in their fertile years are now considering cryopreservation to preserve their fertility after cancer treatment.

Precautions

Before one go for the sperm banking one should go under several medical tests such as: HIV I-II, Hepatitis-C virus, Hepatitis-B surface antigen, HTLV-I, and Syphilis. Screening of all these medical tests is before preserving the sperms as they plays a quintessential role on the development of the child and also determines the success rate of the in vitro fertilizations. Hence this technique of sperm banking or preserving the semen or sperm has proved really useful to the infertile as well as the diseased ones.

11.3 IN-VITRO FERTILIZATION (IVF)

In-Vitro Fertilization (IVF) is a type of Assistive Reproductive Technology (ART) which involves retrieving eggs from a woman's ovaries and fertilizing them with sperm in a laboratory dish. This fertilized egg is known as an embryo which can then be frozen for storage or transferred to a woman's uterus.

Significance of IVF

IVF is a blessing for the people with following problems, (Refer Figure 11.1):

- Women with ovulation disorders, premature or ovarian failure, uterine fibroids
- Blocked or damaged male or female ducts
- Women who have had their fallopian tubes removed
- Male factor infertility including decreased sperm count or sperm motility
- Incompatibility between the sperm and the milieu of the egg or the reproductive tract
- Unexplained infertility

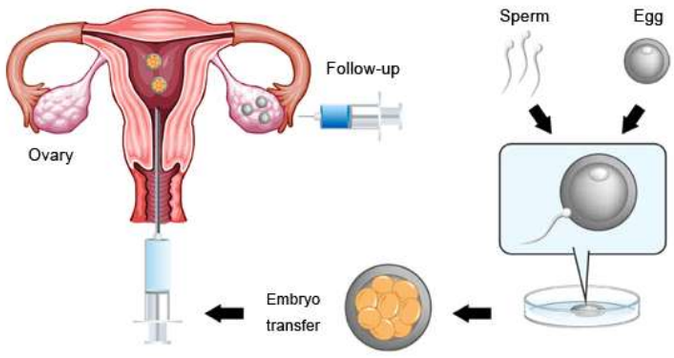

Fig. 11.1 *Diagram Showing IVF Technique*

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Procedure of Performing In-Vitro Fertilization

- **Ovarian Hyper Stimulation:** The process of IVF requires multiple eggs because some eggs will not develop or fertilize after retrieval. Proper examination of ovaries is done through transvaginal ultrasound and a hormone level is checked through blood testing. For preliminary maturation of follicles, ovaries are hyperstimulated to produce mature oocytes by injecting gonadotropins approximately for 10 days. Ovarian hyperstimulation also includes suppression of spontaneous ovulation, (Refer Figure 11.2) for which two main methods are available:
  - **GnRH Agonist Protocol:** It is usually longer. In a standard long GnRH agonist protocol the day when hyperstimulation treatment is started and the expected day of later oocyte retrieval can be chosen to conform to personal choice.
  - **GnRH Antagonist Protocol:** In this case, it must be adapted to the spontaneous onset of the previous menstruation. GnRH antagonist protocol has a lower risk of ovarian hyperstimulation syndrome (OHSS), which is a life-threatening complication. It is shoeter then previous protocol.
- **Final Maturation Induction:** When the ovarian follicles have reached a certain degree of development, induction of final oocyte maturation is performed, generally by an injection of human chorionic gonadotropin (HCG). Commonly, this is known as the 'trigger shot.' HCG acts as an analogue of luteinising hormone and ovulation would occur between 38 and 40 hours after a single HCG injection, but the egg retrieval is performed at a time usually between 34 and 36 hours after HCG injection; that is, just before the rupture of follicles.
- **Egg Retrieval:** The eggs are retrieved from the patient using a transvaginal technique called transvaginal oocyte retrieval, involving an ultrasound-guided needle piercing the vaginal wall to reach the ovaries. Through this needle follicles can be aspirated and the follicular fluid is sent to an embryologist to identify ova. It is common to remove between ten and thirty eggs. The retrieval procedure usually takes between 20 and 40 minutes, depending on the number of mature follicles and is usually done under conscious sedation or general anaesthesia.
- **Egg and Sperm Preparation:** In the laboratory, the identified eggs are stripped of surrounding cells and prepared for fertilisation. An oocyte selection may be performed prior to fertilisation to select eggs with optimal chances of successful pregnancy. Semen samples are collected 2 hours before the oocytes are retrieved. Semen is prepared for fertilisation by removing inactive cells and seminal fluid in a process called sperm washing and thus, the sperms are capacitated.

- **Co-Incubation:** The sperm and the egg are incubated (12-18 h) together at a ratio of about 75,000:1 in a culture media for the actual fertilisation to take place. In certain situations, such as low sperm count or motility, a single sperm may be injected directly into the egg using IntraCytoplasmic Sperm Injection (ICSI). The fertilised egg is passed to a special growth medium and left for about 48 hours until the egg consists of six to eight cells.
- **Embryo Culture and Selection:** The main durations of embryo culture are until cleavage stage (day two to four after coincubation) or the blastocyst stage (day five or six after coincubation). To optimise pregnancy rates, the embryo quality is determined by morphological scoring system.
- **Embryo Transfer:** Embryos are graded by the embryologist based on the number of cells, evenness of growth and degree of fragmentation. The number to be transferred depends on the number available, the age of the woman and other health and diagnostic factors. Different countries follow different regulations for embryo transfer. In countries, such as Canada, UK, Australia and New Zealand, a maximum of two embryos are transferred except in unusual circumstances. According to HFEA regulations, a woman over 40 may have up to three embryos transferred in UK, whereas in the USA, younger women may have many embryos transferred based on individual fertility diagnosis. The embryos judged to be the 'best' are transferred to the patient's uterus through a thin, plastic catheter, which is inserted through her vagina and cervix. Several embryos may be passed into the uterus to improve chances of implantation and pregnancy.
- **Adjunctive Medication:** In order to attain good success rate in IVF certain supporting procedures are followed like Luteal Support (LS). LS is the administration of medication, generally progesterone, progestins or GnRH agonists, to increase the success rate of implantation and early embryogenesis; thereby complementing and/or supporting the function of the corpus luteum.

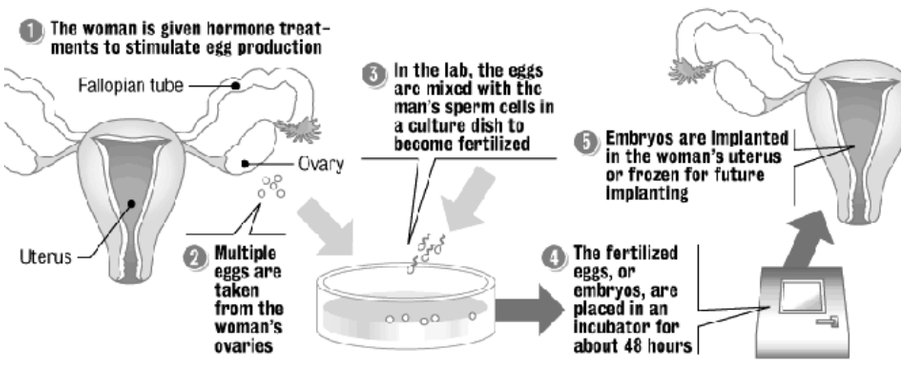

Fig. 11.2 *Procedure of IVF*

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11.3.3 Risks Associated with IVF

- **Multiple Births:** The major complication of IVF is the risk of multiple births. This is directly related to the practice of transferring multiple embryos at embryo transfer. Multiple births are related to increased risk of pregnancy loss, obstetrical complications, prematurity and neonatal morbidity with the potential for long term damage. Strict limits on the number of embryos that may be transferred have been enacted in some countries (for example, Britain, Belgium) to reduce the risk of high-order multiples (triplets or more), but are not universally followed or accepted. Another risk is spread of infectious disease (such as Hepatitis B, HIV/AIDS).
- **Other Risks to the Egg Provider/Retriever:** A risk of ovarian stimulation is the development of ovarian hyperstimulation syndrome, particularly if HCG is used for inducing final oocyte maturation. This results in swollen, painful ovaries. It occurs in 30% of patients. Mild cases can be treated with over the counter medications and cases can be resolved in the absence of pregnancy. In moderate cases, ovaries swell; fluid gets accumulated in the abdominal cavities and may have symptoms of heartburn, gas, nausea or loss of appetite. In severe cases patients have sudden excess abdominal pain, nausea, vomiting and will result in hospitalisation. During egg retrieval, there is a small chance of bleeding, infection and damage to surrounding structures like bowel and bladder (transvaginal ultrasound aspiration) as well as difficulty in breathing, chest infection, allergic reactions to medication, or nerve damage (laproscopic techniques). Ectopic pregnancy may also occur if a fertilised egg develops outside the uterus, usually in the fallopian tubes and requires immediate destruction of the foetus.
- **Birth Defects:** Infants resulting from IVF (with or without ICSI) have a relative risk of birth defects of 1.32% compared to naturally conceived infants. The birth defects include septal heart defects, cleft lip with or without cleft palate, oesophageal atresia, and anorectal atresia. In 2017 studies revealed that there is prevalence of Cerberal Palsy in offsprings produced through in vitro fertilization technique. Cereberal palsy is a congenital neurological disorder which affects the movement and the muscle coordination of the child, in many cases vision, hearing and sensation are also get affected. In total of 211,660 live births were incuded in study and prevalence of cereberal palsy was increased in children born after ART (7.2 /1000 live births compared with natural conceive births 2.5/1000.

Side Effects of IVF

Side effects of IVF may include: Discharge of fluid may be clear or blood-tinged after the procedure from vagina, Mild cramping, Constipation, Breast tenderness, Nausea or vomiting, Decreased urinary frequency, Shortness of breath, Faintness, and Severe stomach pains and bloating.

11.4 ARTIFICIAL INSEMINATION (AI)

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Artificial insemination is an assisted reproductive method that is widely used to combat infertility in couples and help them to conceive and enjoy the parenthood. Main objective behind using this technique is to increase the proportion of male gametes inside the genitalia of female, (Refer Figure 11.3). The effectiveness of artificial insemination has been clearly established in specific subsets of infertile patients, such as those with idiopathic infertility, infertility related to a cervical factor, or mild male factor infertility. An accepted advantage of artificial insemination is that it is generally less expensive and invasive than other Assisted Reproductive Technology (ART) procedures. Unexplained Infertility (i.e., idiopathic) is the infertility which is diagnosed after all known etiologies of infertility. In these cases, semen analyses are normal but still there is no evidence of any female causes of infertility, such as ovulation defects, tubal factor, endometriosis and cervical factor. The average incidence of unexplained infertility is approximately 15% among infertile couples. In couples with unexplained infertility, partner IUI has been demonstrated to improve pregnancy rates when used in conjunction with superovulation.13% in a meta-analysis of almost 1000 superovulation cycles for unexplained infertility, partner IUI was found to almost double pregnancy rates (20%) as compared to timed intercourse alone (11%).

AI is by far the most common method of breeding of intensively kept dairy cattle. In developed countries, advances in artificial insemination already have a major impact on livestock improvement programs. AI speeds up genetic progress, reduces the risk of disease transmission and expands the number of animals that can be bred from a superior parent. The acceptance of AI technology worldwide provided the impetus for developing other technologies, such as cryopreservation and sexing of sperm, estrous cycle regulation and embryo harvesting, freezing, culture & transfer and cloning.

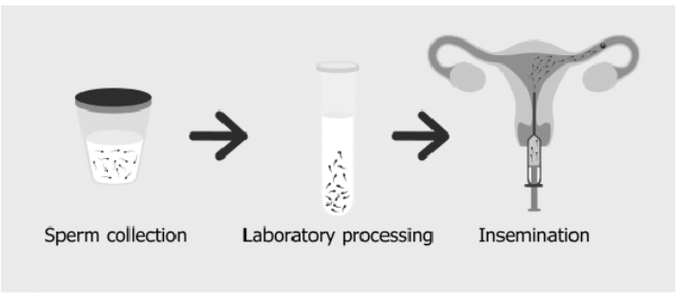

Fig. 11.3 *Schematic Diagram of Artificial Insemination*

Artificial insemination is a technique in which sperms are collected from the male, processed, stored and artificially introduced into the female reproductive tract at proper time for the purpose of conception. AI is most common method of breeding intensively kept domestic livestock, such as dairy cattle, pigs and turkeys.

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AI is increasing in horses, beef cattle and sheep; and has been reported in other domestic species, such as dogs, goats, deer and buffalo. It has also been used occasionally in conservation breeding of rare or endangered species, for example, primates, elephants and wild felids. Artificial Insemination as a means of livestock improvement are now accepted and utilized worldwide. Hafez (1980) pointed out that artificial insemination is the most important single technique ever devised for the genetic improvement of animals. This is possible because, a few highly selected males produce enough spermatozoa to inseminate thousands of females per year, whereas only relatively few progeny per selected female can be produced per year even by embryo transfer. The increased use of outstanding proven sires to enhance production potentials, control genital diseases transmitted through natural service which aid in animal improvement results from the expanding use of Artificial insemination. AI gun is an instrument used for artificial insemination and embryo transfer. It has a very narrow diameter stainless steel plunger with a knob handle. The plunger is fitted into a plastic straw from which the semen or embryo is expelled.

History

Artificial insemination has been used in clinical medicine for more than 200 years for the treatment of infertile couples. In 1780, Lazzaro Spallanzani first successfully reported first use of 'Artificial Insemination'. He performed his experiment with dog, and inseminate the bitch during the heat period. Sixty-two days later she gave birth to three pups. He is also called 'Father of Modern Artificial Insemination'. In 1922, E.I. Ivanoff, a leading Russian investigator and a pioneer in artificial insemination, was the first man to undertake successfully the AI of cattle and sheep. Ivanoff worked with stud farms. He obtained successful results with 10 cows. Danish veterinarians (1937) developed the first rectovaginal/cervical fixation method of artificial insemination. In India, first time, AI was done by Sampat Kumaran (1939) at 'Palace Dairy Farm Mysore'. He inseminated large number of Halliker cows with semen of Holstein Friesian and got 33 cows pregnant. Philips and Lardy (1940) developed egg yolk phosphate diluter for preserving fertility and motility of refrigerated bull spermatozoa. Salisbury et al. (1941) developed egg yolk citrate diluter. The first buffalo calf through AI (1943) was born at the Allahabad Agricultural Institute. Polge, Smith, and Parkes (1949) discovered cryoprotective effect of glycerol in frozen semen technology.

Today, artificial insemination is frequently used in the treatment of couples with various causes of infertility, including ovulatory dysfunction, cervical factor infertility and unexplained infertility as well as those with infertility caused by endometriosis, male and immunologic factors. Artificial insemination with donor semen has become a well-accepted method of conception. Artificial insemination with donor semen has become a well-accepted method of conception and widely available terms like homologous artificial insemination and heterologous artificial insemination were used to differentiate these two alternative sources. However, the use of these biomedical terms in this manner is at variance with their scientific

meaning, where they denote different species or organisms (as in, homologous and heterologous tissue grafts). In the latter half of the 20th century, the terms Artificial Insemination Donor (AID) and Artificial Insemination Husband (AIH) found common use. However, the widespread use of the acronym AIDS for Acquired ImmunoDeficiency syndrome resulted in the replacement of AID with Therapeutic Donor Insemination (TDI). An analogous alternative term for AIH has not evolved, probably in part because of the increasingly common situation where the woman's partner is not her legal husband.

Donor Evaluation

Each donor must be screened for risk factors and clinical evidence of communicable diseases, including: Human Immunodeficiency Virus Types 1 and 2; Human T-Lymphotropic Virus Types I and II; Hepatitis B and C; Cytomegalovirus; Human Transmissible Spongiform Encephalopathy (Creutzfeldt-Jakob Disease); *Treponema pallidum*; *Chlamydia trachomatis*; and *Neisseria gonorrheae.*

Types of Insemination

- **Intra Uterine Insemination (IUI):** Intra Uterine Insemination (IUI) is a fertility treatment that involves placing sperm inside a woman's uterus to facilitate fertilization. The goal of IUI is to increase the number of sperm that reach the fallopian tubes and subsequently increase the chance of fertilization.
- **Intra Cervical Insemination:** Intra Cervical Insemination (ICI) simulates the ejaculation of semen by the penis into the vagina during intercourse. It is painless and is the easiest and most common method of artificial insemination. It is the technique used in most home and many practitioner artificial inseminations, and because of its ease of use, is a method commonly used by single women and lesbians purchasing semen on-line from sperm banks. ICI involves the introduction of unwashed or raw semen into the vagina at the entrance to the cervix, usually by means of a needleless syringe. Semen supplied by a donor through a sperm bank which has been prepared for IUI use may be used instead of raw, unwashed semen. The procedure is commonly used in home, self-insemination and practitioner insemination procedures and for insemination where semen is supplied by private donors.
- **Transcervical Cannulation (Intratubal Insemination):** Intra Tubal Insemination (ITI) involves injection of washed sperm into the fallopian tube, although this procedure is no longer generally regarded as having any beneficial effect compared with IUI. ITI however, should not be confused with GIFT, where both eggs and sperm are mixed outside the woman's body and then immediately inserted into the fallopian tube where fertilization takes place.
- **Intrauterine Tuboperitoneal Insemination (Intraperitoneal Insemination):** Intrauterine Tuboperitoneal Insemination (IUTPI) involves

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injection of washed sperm into both the uterus and fallopian tubes. The cervix is then clamped to prevent leakage to the vagina, best achieved with a specially designed Double Nut Bivalve (DNB) speculum. The sperm is mixed to create a volume of 10ml, sufficient to fill the uterine cavity, pass through the interstitial part of the tubes and the ampulla, finally reaching the peritoneal cavity and the Pouch of Douglas where it would be mixed with the peritoneal and follicular fluid. IUTPI can be useful in unexplained infertility, mild or moderate male infertility, and mild or moderate endometriosis. In non-tubal sub fertility, fallopian tube sperm perfusion may be the preferred technique over intrauterine insemination.

- **Fallopian Tube Sperm Perfusion:** Studies on the dynamics of sperm transport have shown that there is a progressive decline in the number of sperm along the length of the female reproductive tract. In the ampulla of a patent tube, a maximum of only 200 sperms are present after intercourse.

However, it has been shown that the number of sperms can be significantly increased with fallopian tube sperm flushes. Taking these observations into consideration, another simple non-invasive method was introduced called Fallopian tube Sperm Perfusion (FSP). This technique was developed to ensure the presence of higher sperm densities in the fallopian tubes at the time of ovulation compared with standard IUI. Fallopian tube sperm perfusion is based on pressure injection of 4ml of sperm suspension with an attempt to seal the cervix to prevent semen reflux. This result in a sperm flushing of the fallopian tubes and an overflowing of inseminate into the pouch of Douglas.

Intra Uterine Insemination (IUI)

Intra Uterine Insemination (IUI) is a form of treatment where sperm are inserted into the uterine cavity around the time of ovulation. IUI can be carried out in a natural cycle, without the use of drugs, or the ovaries may be stimulated with oral anti-oestrogens or gonadotrophins. When oral anti-oestrogens are used to stimulate a cycle, a woman will have to take a course of tablets for 5 days. When gonadotrophins are used to stimulate a cycle, the woman usually receives a course of daily fertility injections for 7 to 10 days. However, the exact duration of stimulation will depend on which day of the cycle it is started. In both circumstances the treatment should be monitored by ultrasound scan to assess the ovarian response. When one to three follicles are seen to have developed to a suitable size, usually with one dominate follicle, then an injection of Human Chorionic Gonadotrophin (HCG) is given which triggers ovulation.

Insemination of prepared sperm will be undertaken 24 to 36 hours later. However, in order to reduce the risk of multiple pregnancies, insemination may not be undertaken if more than three follicles have developed or two or more mature follicles are seen. IUI has been used in people with: unexplained infertility; mild endometriosis; mild male factor infertility; disability (physical or psychological) preventing vaginal sexual intercourse; conditions that require specific consideration

in relation to methods of conception (such as after sperm washing in a couple where the male is HIV positive); and as part of donor insemination.

Success Rate of IUI or AI

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The actual per cycle fecundity rate with donor IUI is dependent on multiple factors. A meta-analysis of seven studies demonstrated that IUI yielded a higher pregnancy rate per cycle than intracervical insemination with donor frozen sperm. Overall, the average live birth rate per cycle of donor IUI is approximately 10%.

- **LH Surge:** A commonly used method for timing of IUI is based on urinary LH measurement. Ovulation occurs 40 to 45hours after the onset of the LH surge. Insemination is thus planned for the day after detection of a rise in urinary LH. This approach offers the simplest and most cost-effective of the indirect methods for predicting ovulation and is just as effective in achieving pregnancy as more complex ones.
- **Ultrasound and Human Chorionic Gonadotropin:** Transvaginal ultrasonography is widely used to monitor the size of the follicles and to assess the timing of ovulation. Follicles become recognizable once they grow to 2 to 3mm in diameter. After 8 mm, linear follicular growth occurs at a rate of approximately 2 to 3mm per day. Ovulation occurs during a natural cycle when the lead follicle reaches 15 to 24mm in size. Injection of HCG can be given to induce predictable ovulation when at least one follicle diameter is between 17 and 21mm. For optimal pregnancy rates, IUI is scheduled 24 to 36hours after the injection.
- **Female Factors that Predict AI Success**: The success of artificial insemination does not only depend on the quality of oocytes and spermatozoa, but also on the receptivity of the endometrium. Studies showed that the presence of uterine anomalies such as endometrial thickness and pattern negatively affected the success of IUI. A study evaluated the role of endometrial volume measurement in predicting the pregnancy rate in women receiving controlled ovarian hyperstimulation and IUI. An endometrial volume of less than 2ml three-dimensional ultrasound on the day of insemination was associated with a poor likelihood of pregnancy. Pregnancy rates after IUI are dependent on ovum pickup and transport. It follows that pregnancy rates after IUI are decreased by other causes of female infertility, including tubal factor and endometriosis. Success of IUI also greatly depends upon the age of the woman and is an indirect indicator for oocyte quality and has a significant effect on the pregnancy rates. An age-related decline in female fecundity has been documented in women undergoing IUI. Successful pregnancy rates decrease after age 35 and reduce dramatically after age 40. However, pregnancies can occur at relatively advanced maternal ages, and satisfactory pregnancy rates can be obtained with IUI among women aged from 40 to 42 years.

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- **Male Factors that Predict AI Success:** Seminal characteristics impart higher chances of initiating a successful pregnancy as a result of IUI than those with abnormal results on semen analysis. This association is probably related to two associated factors. First, an abnormal semen analysis is often associated with an impaired fertilization capacity. Secondly, pregnancy rates positively correlate with the total number of motile sperm recovered for IUI and this number is often lower in men with abnormal results on semen analysis. Other factors such as Sperm count and sperm physiology also plays a vital role in the success rate of IUI.

Sperm Preparation for AI

Prior to the Sperm preparation, certain methods are used to process semen samples so that viable sperm are separated from seminal plasma. This is necessary before IUI, to avoid the consequences of intrauterine injecting of semen plasma proteins and prostaglandins. Although seminal plasma protects the spermatozoa from stressful conditions such as oxidative stress, it also contains factors that inhibit the fertilizing ability of the spermatozoa and reduce the induction of capacitation. Sperm preparation involves removing the seminal plasma efficiently and quickly and eliminating dead sperm, leukocytes, immature germ cells, epithelial cells and microbial contamination. Some of the ways usually used by the physicians before the administration of semen inside the woman are given below:

- **Density Gradient Centrifugation:** The density gradient method is a sperm washing method that removes both semen fluid and separates living sperm from other material, including dead sperm cells, white blood cells, and bacteria. For this method a density gradient is prepared by layering suspensions of different concentrations of coated colloidal silica particles (for example, Percoll) in a conical centrifuge tube, with the higher concentrations more superior. The liquified semen sample is placed over the upper layer and the tube is centrifuged. The supernatant is removed from the pellet and the process is repeated. The final pellet is resuspended in wash media and used for AI. Density gradient sperm washes take approximately 1 hour.
- **Glass Wool Filtration:** The glass wool filtration method is another method for removing seminal fluid and separating living sperm from other cellular material after sperm has been washed. For this technique: The semen samples are first diluted with wash media and centrifuged in a manner similar to sperm washing. The resulting pellet is resuspended in wash media and placed on glass wool columns, created by inserting glass wool into the barrel of a 3ml syringe. The washed sperm solution is allowed to filter through the column by gravity, and the filtrate is collected for AI.

Need for Artificial Insemination

In the following situations the artificial insemination become beneficial: Disorders of semen density and motility; Mild oligospermia; Medical conditions that could endanger asthenozoospermia, the patient's life teratozoospermia); Partner's with hereditable disease; Cervical neoplasia Ejaculatory; Mild endometriosis ; Ovulatory dysfunction; Chemotherapy or radiotherapy; Coexisting multiple infertility etiologies; Pelvic surgery; and Older age woman Unexplained Fertility.

Risks and Complications Associated with AI

Following are the risks and complications associated with AI:

- **Pelvic Infections:** Pelvic infection is a kind of complication which is associated with the equipments used while performing an AI procedure. These symptoms are self-limiting and should resolve within hours of the procedure. Continued discomfort can be an indication of a developing pelvic infection, which has been estimated to occur in less than 2 per 1000 AI procedures. Early diagnosis and treatment is essential in these rare cases to minimize the risk to the patient, particularly with subsequent decreased fertility.
- **Vasovagal Reaction:** Vasovagal reactions can occur as a result of manipulation of the cervix. The resulting vasodilation and decreased heart rate can lead to hypotension, most commonly manifest by diaphoresis in a supine patient. Sitting or standing increases the risk of syncope, which is unlikely to cause supine. Persistent symptomatic vasovagal reactions in a supine patient will often respond to the patient crossing her legs. More severe cases might require treatment with intramuscular atropine injection (0.5 mg) to cure the pain.
- **Allergic Reaction:** Allergic reactions, including anaphylaxis, can occur after IUI in response to potential allergens in the wash media. Reactions have been reported to both the bovine serum albumin and antibiotics (penicillin and streptomycin) commonly used in the wash media. Of these, penicillin allergies are the most common in the general population. Allergic reactions after IUI can range from a mild skin rash to life-threatening anaphylaxis with laryngeal edema, bronchospasm and hypotension. For the rare patient experiencing an allergic reaction after IUI, the use of wash media free of albumin and antibiotics is advised.

AI in Different Species

- **AI in Cattle:** In cattle, frozen semen doses are used most widely in Europe and North America, since there are well-established protocols for cryopreserving bull semen, (Refer Figure 11.4). Semen doses typically contain approximately 15 million motile spermatozoa. In New Zealand, however, fresh semen doses are used instead, with AI occurring within 24h of semen collection.

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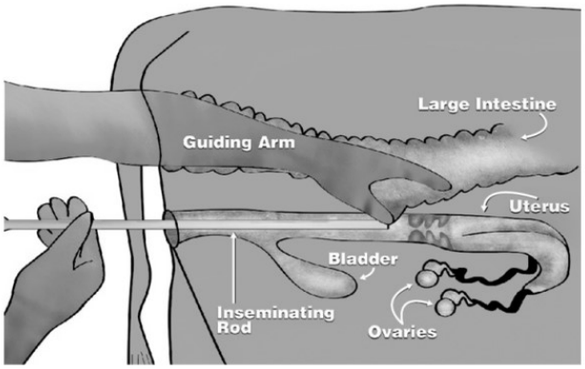

Fig. 11.4 *Artificial Insemination in Cattle*

- **AI in Pigs:** The porcine AI industry uses liquid semen that has been stored for one to several days at 16-18°C. In contrast, AI with cryopreserved boar spermatozoa results in lower farrowing rates and litter sizes than with cooled, stored spermatozoa, making the use of frozen-thawed sperm doses unattractive for commercial pig breeders.
- **AI in Horses**: AI has increased in horses in the last 25 years. Initially, fresh semen was used for AI shortly after semen collection, but nowadays the use of cooled semen has largely replaced fresh semen. The extended semen is cooled to approximately 5°c, and transported in insulated containers, together with a cold pack. The fertility of the cooled semen is maintained for approximately 24h. Frozen semen doses are used infrequently, although this trend may change with the development of better freezing protocols.
- **AI in Sheep and Goat:** AI in sheep and goats is traditionally performed with fresh or cooled spermatozoa, with acceptable fertility results. However, use of foreign breeds, genetic improvement and the use of 'safe' semen from other countries requires the use of frozen semen, to enable analyses for contaminants or diseases in the 'donor' male to be completed before the semen doses are used for AI. Although the post-thaw motility of frozen semen from goats and sheep is usually considered acceptable, low fertility has been associated with its use in AI, mainly owing to a shortened lifespan of the spermatozoa.

Check Your Progress

1. What is sperm banking also referred as?
2. What is the technical name for sperm banking?
3. What percent of couples does infertility affects globally?
4. What does gene knockout do?

11.5 EMBRYO TRANSFER

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An embryo transfer is the last part of the In-Vitro Fertilization (IVF) process. During IVF, fertility medications are used to stimulate the ovaries into releasing healthy eggs. These eggs are then removed from a woman's ovaries and fertilized in a lab. Once the fertilized eggs have multiplied, the embryos are transferred to the woman's uterus. For a pregnancy to begin, the embryo must then attach itself to the wall of her womb or uterus.

Need of Embryo Transfer

IVF and embryo transfer is needed in cases where natural fertilization is not an option or has difficulty in occurring. There are many reasons for embryo transfer, including:

- **Ovulation Disorders**: If ovulation is infrequent, fewer eggs are available for successful fertilization.
- **Damage to Fallopian Tubes**: The Fallopian tubes are the passageway through which the embryos travel to reach the uterus. If the tubes become damaged or scarred, it is difficult for fertilized eggs to safely reach the womb.
- **Endometriosis**: When tissue from the uterus implants and grows outside of the uterus. This can affect how the female reproductive system works.
- **Premature Ovarian Failure**: If the ovaries fail, they do not produce normal amounts of estrogen or release eggs regularly.
- **Uterine Fibroids**: Fibroids are small, benign tumors on the walls of the uterus. They can interfere with an egg's ability to plant itself in the uterus, preventing pregnancy.
- **Genetic Disorders**: Some genetic disorders are known to prevent pregnancy from occurring.
- **Impaired Sperm Production**: In men, low sperm production, poor movement of the sperm, damage to the testes, or semen abnormalities are all reasons natural fertilization may fail.

Around 2 or 3 days before the embryo transfer, the doctor will choose the best eggs to transfer to the womb. There are many processes available to aid selection, though non-invasive methods such as metabolomic profiling are being tested. Metabolomic profiling is the process of selecting the most beneficial eggs based on a number of different factors. This could limit the need for invasive procedures in the future. These eggs will then be fertilized in a lab and left to culture for 1-2 days. If many good quality embryos develop, the ones that are not going to be transferred can be frozen.

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Process of an Embryo Transfer

The embryo transfer process is similar to the process for a pap smear. The doctor will insert a speculum into the woman's vagina to keep the vaginal walls open. Using ultrasound for accuracy, the doctor will then pass a catheter through the cervix and into the womb. From there, the embryos are passed through the tube and into the womb. The processes are usually pain free and rarely require any sedatives. Some women may feel discomfort as a result of having the speculum inserted or from having a full bladder, which is required for ultrasound. The process is short and the bladder can be emptied immediately after (Refer Figure 11.5).

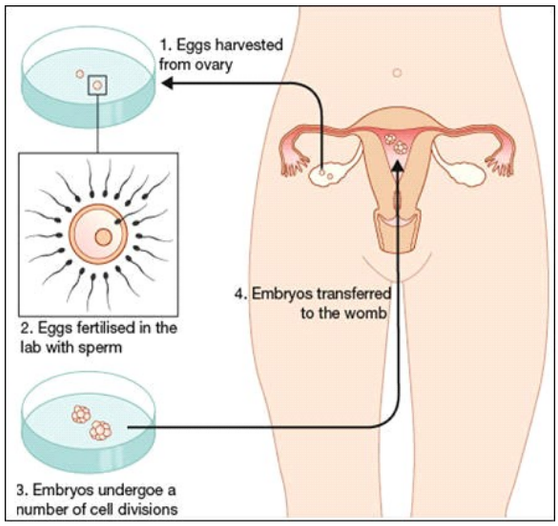

Fig. 11.5 *Embryo Transfer*

Types of Embryo Transfer

- **Fresh Embryo Transfer**: Once eggs have been fertilized, they are cultured for 1-2 days. The best embryos are chosen to transfer directly to the woman's uterus.
- **Frozen Embryo Transfer**: Any healthy embryos that were not used in the first transfer can be frozen and stored for future use. These can be thawed and transferred to the uterus.
- **Blastocyst Embryo Transfer**: If many healthy embryos develop after the fertilization, it is common to wait to see if the embryos develop into blastocysts. According to a study, blastocyst embryo transfer has a higher success rate than the standard embryo transfer on day 3. However, another recent study suggests that it may pose risks later in pregnancy and should not always be recommended.
- **Assisted Hatching (AH)**: Studies have also found that the process of assisted hatching (weakening the outer layer of the embryo before it is transferred to the uterus) does not improve pregnancy and implantation

rates in women who are having fresh embryos transferred. The researchers noted, however, that women having frozen embryos implanted do benefit from having their embryos treated in this way. The number of fresh embryos to be transferred varies according to the woman's age and outlook. In many cases, no more than two embryos will be used. For women under the age of 35 with an excellent chance of pregnancy, doctors will consider using just one embryo. A recent study showed that single embryo transfer in women less than 38 years of age reduces the risk of multiple births, yet does not seem to affect live birth rates. This is important to note, as many doctors recommend using multiple embryos to ensure pregnancy.

Success Rates of Embryo Transfers

Studies suggest that there may only be a small difference in success rates between fresh and frozen embryos used in IVF. The rate of success for embryo transfers may vary based on the transfer method used. According to a study, there is no statistical difference between using fresh and frozen embryos. Embryo transfers using fresh embryos had a 23% pregnancy rate, whereas frozen embryos had an 18 percent pregnancy rate. The study showed that frozen embryos could also be used for additional embryo transfers where fresh embryos could not. If the chance for pregnancy is low, doctors may consider freezing additional embryos for a second attempt at embryo transfer at a later date. Individual success rates can vary and may depend upon the cause of infertility, ethnic backgrounds and genetic disorders.

Risks and Precautions of Embryo Transfers

Risks are mostly related to increased hormonal stimulation, causing an increased risk such as a blood clot blocking a blood vessel. The woman can also experience bleeding, changes in her vaginal discharge, infections and complications of anesthesia if it is used. The risk of a miscarriage is about the same as in natural conception. The greatest risk of embryo transfer is the chance of multiple pregnancies. This occurs when multiple separate embryos attach to the uterus. This may increase the risk of stillbirth and children born with disabilities and is more common in pregnancies due to IVF than natural conception.

11.6 TEST-TUBE BABY

Test tube baby is a term that refers to a child that is conceived outside the women's body by a scientific process known as in vitro fertilization or IVF treatment, (Refer Figure 11.6).This entire process is done in a laboratory. In this process the eggs are taken from the mother's ovary and fertilised by the sperms from the father. The fertilised egg is cultured for 2–6 days and allowed to divided 2-4 times inside a test tube (hence the name test tube baby) These eggs are then returned back to the mother's uterus where it can be developed normally, this is done with the

intention to establish a successful pregnancy. Test tube baby procedure has greatly helped women having infertility problems that are untreatable to give birth to healthy babies.

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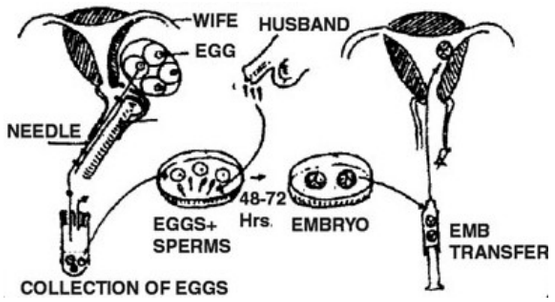

Fig. 11.6 *Diagram Showing Methodology for Test-Tube Baby*

Test tube baby is a term sometimes used by the media to refer to children conceived with IVF. Despite the name, 'test tube babies' are not developed in a test tube. Test tubes are not part of the modern IVF process at all. With IVF, the egg is fertilized in a petri dish. (Not a test tube.) When the embryo is between three and five days old, it is transferred to the uterus. To be clear, the embryo does *not* develop into a fetus in the lab. That idea belongs to the realms of science fiction. The embryo transferred is a collection of living and developing cells, not what anyone would think of as a 'fetus'. The term test tube baby was first used in the 1930s. Then, it was used to refer to AI not IVF. Artificial insemination is when specially washed semen is directly transferred into a woman's uterus via the cervix. It is an *in vivo* fertilization in the body and not *in vitro*, in the lab, like IVF, (Refer Figure 11.7.) An early reference to the phrase 'test tube baby' is found in a book published in 1934 by Panurge Press, written by Dr. Hermann Rohleder. The book, entitled *Test Tube Babies: A History of the Artificial Impregnation of Human Beings*, is described as, 'including a detailed account of its technique, together with personal experiences clinical cases, a review of the literature, and medical and legal aspects involved'. When the first human egg was fertilized outside of the body in 1944, the term test tube baby began to refer to IVF babies. Louise Joy Brown, the world's first IVF baby, is still frequently referred to as the world's 'first test tube' baby.

Procedure for Test-Tube Baby

Now let us understand the steps involved in test tube baby process. The steps for test tube baby break in four phases. People planning for IVF treatment should know the test tube baby procedure in depth.

Step 1. Egg Stimulation: Patient is given fertility medications to stimulate the production of egg. To increase the success rate of the treatment multiple eggs are needed. Usually it not advisable to rely on single egg for which the doctor gives fertility drug to increase the production of eggs. Depending upon the patient's response medications are advised. There are various protocols for egg stimulation.

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Egg stimulation is guided by Transvaginal ultrasound to examine ovaries, blood samples and to determine the level of hormones.

Step 2. Egg Retrieval: Imaging ultra sound is used to retain ovarian follicles with the help of hollow needle. Retrieval of egg is followed with minor surgery. Complete procedure takes about half an hour. The follicular fluids are carefully seen by embryologist to trace the proof of available eggs. After the entire procedure eggs are preserved in incubator till insemination.

Step 3. Culture of Fertilization and Embryo: For insemination male sperm sample is collected. Eggs are mixed with the sperms and stored in laboratory.If there are chances of low probability of fertilization, ICSI can be considered. To enable fertilization single sperm is infused in the egg. Fertilized eggs are considered as embryos only after the confirmation by an embryologist.

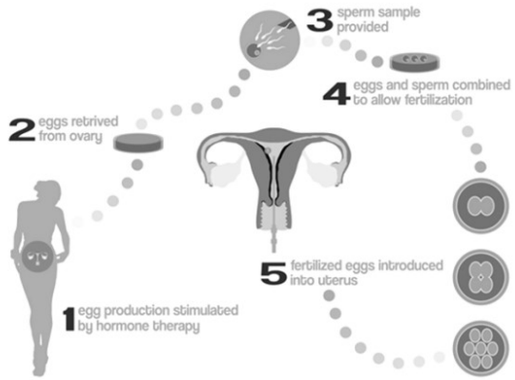

Fig. 11.7 *Step-by-Step Procedure of IVF and Test Tube Baby Technique*

Step 4. Transfer of Egg and Quality of Embryo: Embryo transfer is the quickest method done in overall treatment Embryo is assessed on the basis of age and quality. Doctor is asked to keep the full history of the patient starting from age to previous treatment, but the end decision is taken by patient itself. Under normal situation, doctor suggests transferring single embryo with blastocyst and rest of them are preserved. Transfer of one high quality embryo decreases the risk of triplets or twins. During transferring, doctor inserts Cather and pushes pre determined embryos in the female uterus. This method is done under guidance of ultra sound. Patient is advised to take rest for 5-6 hours after it. After this method, pregnancy test is conducted to know the exact situation.

Check Your Progress

5. What are the two types of CPA?
6. What is short-term semen cryobanking?
7. What is the the last part of the In-Vitro Fertilization (IVF) process?
8. What kind of semen does the porcine AI industry use?

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11.7 GENE KNOCK-IN

In molecular biology, a knock-in (or gene knock-in) refers to a genetic engineering method that involves the one-for-one substitution of DNA sequence information in a genetic locus or the insertion of sequence information not found within the locus. Typically, this is done in mice since the technology for this process is more refined and there is a high degree of shared sequence complexity between mice and humans. The difference between knock-in technology and traditional transgenic techniques is that a knock-in involves a gene inserted into a specific locus, and is thus a 'targeted' insertion. A common use of knock-in technology is for the creation of disease models. It is a technique by which scientific investigators may study the function of the regulatory machinery (e.g. promoters) that governs the expression of the natural gene being replaced. This is accomplished by observing the new phenotype of the organism in question. The BACs and YACs are used in this case so that large fragments can be transferred.

Gene Knok-In Techniques

Gene knock-in originated as a slight modification of the original knockout technique developed by Martin Evans, Oliver Smithies, and Mario Capecchi. Traditionally, knock-in techniques have relied on homologous recombination to drive targeted gene replacement, although other methods using a transposon-mediated system to insert the target gene have been developed, (Refer Figure 11.8). The use of *loxP* flanking sites that become excised upon expression of Cre recombinase with gene vectors is an example of this. Embryonic stem cells with the modification of interest are then implanted into a viable blastocyst, which will grow into a mature chimeric mouse with some cells having the original blastocyst cell genetic information and other cells having the modifications introduced to the embryonic stem cells. Subsequent offspring of the chimeric mouse will then have the gene knock-in. Gene knock-in has allowed, for the first time, hypothesis-driven studies on gene modifications and resultant phenotypes. Mutations in the human p53 gene, for example, can be induced by exposure to benzo(a)pyrene and the mutated copy of the p53 gene can be inserted into mouse genomes. Lung tumors observed in the knock-in mice offer support for the hypothesis of BaP's carcinogenicity. More recent developments in knock-in technique have allowed for pigs to have a gene for green fluorescent protein inserted with a CRISPR/Cas9 system, which allows for much more accurate and successful gene insertions. The speed of CRISPR/Cas9-mediated gene knock-in also allows for biallelic modifications to some genes to be generated and the phenotype in mice observed in a single generation, an unprecedented time frame.

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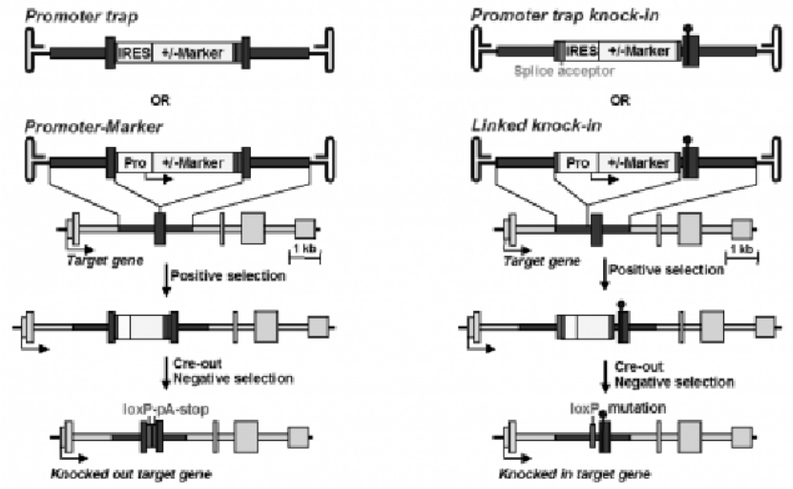

Fig. 11.8 *Schematic Diagram of Target Gene Knock-Out and Knock-In*

Gene Knock-In V*s.* Knock-Out

Knock-in technology is different from knock-out technology in that knock-out technology aims to either delete part of the DNA sequence or insert irrelevant DNA sequence information to disrupt the expression of a specific genetic locus, (Refer Figure 11.9). Gene knock-in technology, on the other hand, alters the genetic locus of interest via a one-for-one substitution of DNA sequence information or by the addition of sequence information that is not found on said genetic locus. A gene knock-in therefore can be seen as a gain of function mutation and a gene knockout a loss of function mutation, but a gene knock-in may also involve the substitution of a functional gene locus for a mutant phenotype that results in some loss of function.

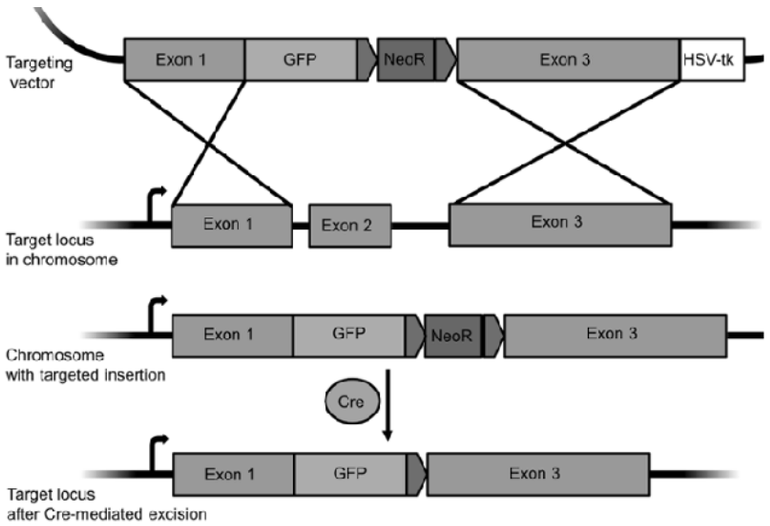

Fig. 11.9 *A Knock-Out and Knock-In Targeting Vector Designed to Insert GFP into a Given Locus*

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Applications of Gene Knock-In

Because of the success of gene knock-in methods thus far, many clinical applications can be envisioned. Knock-in of sections of the human immunoglobulin gene into mice has already been shown to allow them to produce humanized antibodies that are therapeutically useful. It should be possible to modify stem cells in humans to restore targeted gene function in certain tissues, for example possibly correcting the mutant gamma-chain gene of the IL-2 receptor in hematopoietic stem cells to restore lymphocyte development in people with X-linked Severe Combined Immuno Deficiency (SCID).

Limitations of Gene Knock-In

While gene knock-in technology has proven to be a powerful technique for the generation of models of human disease and insight into proteins *in vivo*, numerous limitations still exist. Many of these are shared with the limitations of knockout technology. First, combinations of knock-in genes lead to growing complexity in the interactions that inserted genes and their products have with other sections of the genome and can therefore lead to more side effects and difficult-to-explain phenotypes. Also, only a few loci, such as the ROSA26 locus have been characterized well enough where they can be used for conditional gene knock-ins, making combinations of reporter and transgenes in the same locus problematic.

The biggest disadvantage of using gene knock-in for human disease model generation is that mouse physiology is not identical to that of humans and human orthologs of proteins expressed in mice will often not wholly reflect the role of a gene in human pathology. This can be seen in mice produced with the ”F508 fibrosis mutation in the CFTR gene, which accounts for more than 70% of the mutations in this gene for the human population and leads to cystic fibrosis. While ”F508 CF mice do exhibit the processing defects characteristic of the human mutation, they do not display the pulmonary pathophysiological changes seen in humans and carry virtually no lung phenotype. Such problems could be ameliorated by the use of a variety of animal models, and pig models (pig lungs share many biochemical and physiological similarities with human lungs) have been generated in an attempt to better explain the activity of the ”F508 mutation.

11.8 GENE KNOCK-OUT

A Gene Knock-Out (KO) is a genetic technique in which one of an organism’s genes is made inoperative (‘knocked out’ of the organism), (Refer Figure 11.10). However, KO can also refer to the gene that is knocked out or the organism that carries the gene knockout. Knockout organisms or simply knockouts are used to study gene function, usually by investigating the effect of gene loss. Researchers draw inferences from the difference between the knockout organism and normal individuals.

The KO technique is essentially the opposite of a gene knock-in. Knocking out two genes simultaneously in an organism is known as a Double Knock-Out (DKO). Similarly the Terms Triple Knock-Out (TKO) and (QKO) are used to describe three or four knocked out genes, respectively. However, one needs to distinguish between heterozygous and homozygous KOs. In the former, only one of two gene copies (alleles) is knocked out, in the latter both are knocked out.

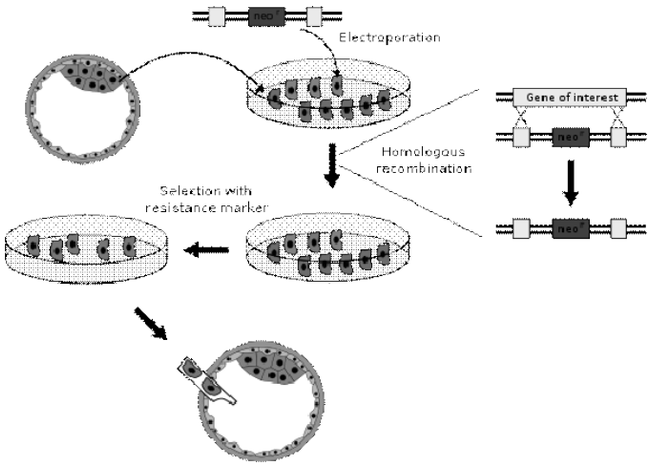

Fig. 11.10 *Diagram Showing Breeding Scheme for Producing Knock-Out Mouse*

Gene Knock-Out Techniques

Knockouts are accomplished through a variety of techniques. Originally, naturally occurring mutations were identified and then gene loss or inactivation had to be established by DNA sequencing or other methods, (Refer Figure 11.11).

- **Homologous Recombination:** Traditionally, homologous recombination was the main method for causing a gene knockout. This method involves creating a DNA construct containing the desired mutation. For knockout purposes, this typically involves a drug resistance marker in place of the desired knockout gene. The construct will also contain a minimum of 2kb of homology to the target sequence. The construct can be delivered to stem cells either through microinjection or electroporation. This method then relies on the cell's own repair mechanisms to recombine the DNA construct into the existing DNA. This results in the sequence of the gene being altered, and most cases the gene will be tranlated into a nonfunctional protein, if it is translated at all. However, this is an inefficient process, as homologous recombination accounts for only $10^{"2}$ to 10^{-3} of DNA integrations. Often, the drug selection marker on the construct is used to select for cells in which the recombination event has occurred.
- **Site-Specific Nucleases:** There are currently three methods in use that involve precisely targeting a DNA sequence in order to introduce a double-stranded break. Once this occurs, the cell's repair mechanisms will attempt

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to repair this double stranded break, often through Non-Homologous (NHEJ), which involves directly ligating the two cut ends together. This may be done imperfectly, therefore sometimes causing insertions or deletions of base pairs, which cause frameshift mutations. These mutations can render the gene in which they occur nonfunctional, thus creating a knockout of that gene. This process is more efficient than homologous recombination, and therefore can be more easily used to create biallelic knockouts, for example, Zink-finger nuclease, TALENs (Transcription Activator-Like Effector Nucleases), CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats).

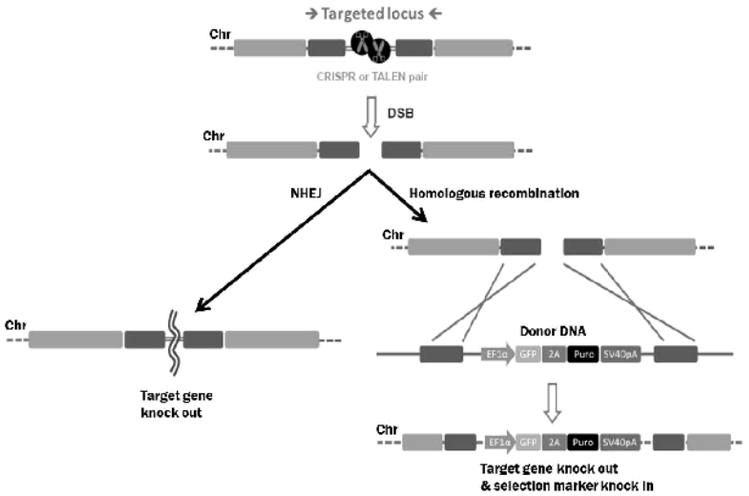

Fig. 11.11 *Diagram Showing Knock-Out by CRISPR vs Knock-Down by SiRNA*

Conditional Knock-Out

A conditional knock-out allows gene deletion in a tissue in a time specific manner. This is required in place of a gene knockout if the null mutation would lead to embryonic death. This is done by introducing short sequences called loxP sites around the gene. These sequences will be introduced into the germ-line via the same mechanism as a Knock-Out. This germ-line can then be crossed to another germline containing Cre-recombinase which is a viral enzyme that can recognize these sequences, recombines them and deletes the gene flanked by these sites.

Uses of Knock-Out

Knock-Outs are primarily used to understand the role of a specific gene or DNA region by comparing the knockout organism to a wild type with a similar genetic background. Knock-Out organisms are also used as screening tools in the development of drugs, to target specific biological processes or deficiencies by using a specific knockout, or to understand the mechanism of action of a drug by using a library of Knock-Out organisms spanning the entire genome, such as in *Saccharomyces cerevisiae*.

Check Your Progress

9. What is a test tube baby?
10. For whom has the preservation of sperms proved to be advantageous?

11.9 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Sperm banking or sperm bank is also referred as cryobank.
2. Gamete cryopreservation is the technical name for sperm banking.
3. Infertility affects an estimated 15% of couples globally.
4. Gene knockout is to remove a gene from the cell's DNA so that it is impossible for it to be expressed.
5. Two types of CPAs are peneteration CPA and nonpeneteration CPA.
6. Short-term semen cryobanking is the depositing, freezing and storage of sperm at a sperm bank for less than one year.
7. An embryo transfer is the last part of the in vitro fertilization (IVF) process.
8. The porcine AI industry uses liquid semen that has been stored for one to several days at 16-18°C.
9. Test tube baby is a term that refers to a child that is conceived outside the women's body by a scientific process known as in vitro fertilization or IVF treatment.
10. Preservation of sperms has proved very advantageous for the cancer patients as the chemotherapy negatively affects the process of spermatogenesis, either transiently or permanently.

11.10 SUMMARY

- A sperm bank, also referred to as a cyrobank, is a facility that collects, freezes, and stores human sperm.
- The sperm kept at a sperm bank is either donated by men to be used by couples seeking sperm donations for artificial insemination or IVF procedures, or is provided by men who want to preserve their own sperm for future use.
- Artificial insemination is a fertility treatment method used to deliver sperm directly to the cervix or uterus in the hopes of getting pregnant.
- Infertility affects an estimated 15% of couples globally, amounting to 48.5 million couples.
- Sperm banking or sperm bank also referred as cryobank is a facility that that collect, freezes, and stores human sperm.

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- Gene knockout is to remove a gene from the cell's DNA so that it is impossible for it to be expressed. There will be absolutely none of the gene product in the cell.
- Sometimes knocking out a gene completely might kill your cell, which doesn't tell you much about what your gene does, other than the fact that your cell needs it.
- Semen for sperm cryopreservation is generally obtained by masturbation.
- The freezing of spermatozoa was first described in the beginning of the 18th century the quality of the sample for cryopreservation.
- Studies on the dynamics of sperm transport have shown that there is a progressive decline in the number of sperm along the length of the female reproductive tract.
- Prior to the Sperm preparation, certain methods are used to process semen samples so that viable sperm are separated from seminal plasma.
- The density gradient method is a sperm washing method that removes both semen fluid and separates living sperm from other material, including dead sperm cells, white blood cells, and bacteria.
- The glass wool filtration method is another method for removing seminal fluid and separating living sperm from other cellular material after sperm has been washed.
- The porcine artificial insemination industry uses liquid semen that has been stored for one to several days at 16-18°C.
- AI in sheep and goats is traditionally performed with fresh or cooled spermatozoa, with acceptable fertility results.
- An embryo transfer is the last part of the in vitro fertilization (IVF) process.
- Risks are mostly related to increased hormonal stimulation, causing an increased risk such as a blood clot blocking a blood vessel.
- Test tube baby is a term that refers to a child that is conceived outside the women's body by a scientific process known as in vitro fertilization or IVF treatment.
- Two chief approaches to artificial insemination exist:Intrauterine Insemination (IUI) and Intra Cervical Insemination (ICI).
- Artificial Insemination (AI) consists of placing sperm, previously selected from a sample, in the woman's uterus.
- The sperm can be provided either by the partner (AIH) or from a sperm bank (AID).
- Test tube baby refers to a term used for a child that is conceived outside the body of a woman by a scientific procedure known as in vitro fertilization, also known as IVF treatment. This entire procedure is done in a laboratory. This is done with the sole intention for establishing and ensuring a successful pregnancy.

- A test-tube baby is the product of a successful human reproduction that results from methods beyond sexual intercourse between a man and a woman and instead utilizes medical intervention that manipulates both the egg and sperm cells for successful fertilization.
- In molecular cloning, a Knock-In (or Gene Knock-In) refers to a genetic engineering method that involves the one-for-one substitution of DNA sequence information in a genetic locus or the insertion of sequence information not found within the locus.
- A Gene Knock-Out (KO) is a genetic technique in which one of an organism's gene is made inoperative ('knocked out' of the organism). However, KO can also refer to the gene that is knocked out or the organism that carries the gene Knock-Out. The KO technique is essentially the opposite of a gene knock-in.
- Sperm banking or sperm bank also referred as cryobank is a facility that that collect, freezes, and stores human sperm. It is a highly effective method of protecting male fertility potential.
- In Vitro Fertilization (IVF) is a type of assistive reproductive technology (ART) which involves retrieving eggs from a woman's ovaries and fertilizing them with sperm in a laboratory dish. This fertilized egg is known as an embryo which can then be frozen for storage or transferred to a woman's uterus.
- Intra Uterine Insemination (IUI) is a form of treatment where sperm are inserted into the uterine cavity around the time of ovulation. IUI can be carried out in a natural cycle, without the use of drugs, or the ovaries may be stimulated with oral anti-oestrogens or gonadotrophins.

11.11 KEY WORDS

- **Artificial insemination:** It is a fertility treatment method used to deliver sperm directly to the cervix or uterus in the hopes of getting pregnant.
- **Homologous recombination:** Traditionally, homologous recombination was the main method for causing a gene knockout. This method involves creating a DNA construct containing the desired mutation.
- **Gene knock-Out (KO):** It is a genetic technique in which one of an organism's genes is made inoperative ("knocked out" of the organism).
- **Test tube baby**: It is a term that refers to a child that is conceived outside the women's body by a scientific process known as in vitro fertilization or IVF treatment.
- **Uterine fibroids:** These are small, benign tumors on the walls of the uterus. They can interfere with an egg's ability to plant itself in the uterus, preventing pregnancy.

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11.12 SELF-ASSESSMENT QUESTIONS AND EXERCISES

Short Answer Questions

1. Name two cryoprotectants used in cryopreservation.
2. Define metabolomic profiling.
3. Who is known as 'father of modern artificial insemination'?
4. Define the process of transvaginal oocyte retrieval.
5. Name the instrument used in the process of artificial insemination.

Long Answer Questions

1. Define IVF and explain the procedure diagrammatically.
2. Explain pros and cons of artificial insemination.
3. Write an essay on embryo transfer and test tube baby.
4. Give an account on knock-in of targeted gene through necessary illustrations.
5. Describe in detail the knockout of targeted gene through necessary diagram.

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BLOCK - IV

EVOLUTION

UNIT 12 EVOLUTION THEORIES: LAMARCKISM AND DARWINISM

Structure

12.0 INTRODUCTION

The term evolution was first used by Herbert Spencer. The evolution has seen defined as a gradual change from one condition to another. In other words, biological evolution is genetic change in a population from one generation to another. According to Darwin, the evolution can be defined as 'Descent with modifications'. Continuous evolution over many generations can result in the development of new varieties and species. Likewise, failure to evolve in response to environmental changes can, and often does, lead to extinction.

The comparison of similarities between organisms of their form or appearance of parts, called their morphology, has long been a way to classify life into closely related groups. This can be done by comparing the structure of adult organisms in different species or by comparing the patterns of how cells grow, divide and even migrate during an organism's development. Strong evidence for evolution comes from the analysis of homologous structures: structures in different species that no longer perform the same task but which share a similar structure. For examples: the forelimbs of a human, cat, whale, and bat all have strikingly similar bone structures. However, each of these four species' forelimbs performs a different task.

Taxonomy is the branch of biology that names and classifies all living things. Scientists use morphological and genetic similarities to assist them in categorizing life forms based on ancestral relationships. For example, orangutans, gorillas, chimpanzees, and humans all belong to the same taxonomic grouping referred to as a family called Hominidae. These animals are grouped together because of similarities in morphology that come from common ancestry (called homology).

In this unit, you will study about Lamarckism and Neo-Lamarckism, Darwinism and Neo-Darwinism, natural selection, variations and evolution of races in detail.

12.1 OBJECTIVES

After going through this this unit, you will be able to:

- Understand the Lamarckism and Neo-Lamarckism
- Discuss the Darwinism and Neo-Darwinism
- Explain about natural selection, variations and evolution of races

12.2 EVOLUTION

The term evolution is derived from Latin words: *e* = from; *volvere* = unrolling or unfolding. The term evolution was first used by Herbert Spencer. The evolution has seen defined as a gradual change from one condition to another. In other words, biological evolution is genetic change in a population from one generation to another. According to Darwin, the evolution can be defined as 'Descent with modifications'. Continuous evolution over many generations can result in the development of new varieties and species. Likewise, failure to evolve in response to environmental changes can, and often does, lead to extinction. The basic concept of organic evolution is continuity of life with constant modifications which takes place due following reason:

- Environmental conditions in nature are unstable and ever changing
- Organisms have an inherent potentiality of changing according to the changing environmental condition called adaptability which leads to evolution of new species
- All the present species had common ancestors at some or the other time of their evolutionary history.

The evolution in which species adapted to different environmental conditions, diversify and modify along many divergent changes known as divergent evolution. However several species or organisms belonging to various regions and groups migrated in to a common environment or habitat and modified and evolved accordingly. This type of evolution is called convergent evolution. The scientific

evidence for evolution comes from many aspects of biology, and includes fossils, homologous structures, and molecular similarities between species.

The study of Fossils provides evidence about the changes in organisms over long periods of time. Modern paleontology began with the work of Georges Cuvier (1769–1832). Cuvier noted that, in sedimentary rock, each layer contained a specific group of fossils. The deeper layers, which he proposed to be older, contained simpler life forms. He noted that many forms of life from the past are no longer present today. In an attempt to explain extinction, Cuvier proposed the idea of 'revolutions' or catastrophism (sudden disaster) in which he speculated that geological catastrophes had occurred throughout the Earth's history, wiping out large numbers of species. Cuvier's theory of revolutions was later replaced by uniformitarian theories. A very large number of fossils have now been discovered and identified. These fossils serve as a time wise record of evolution. The fossil record provides examples of transitional species that demonstrate ancestral links between past and present life forms. One such transitional fossil is *Archaeopteryx*, an ancient organism that had the distinct characteristics of a reptile (such as a long, bony tail and conical teeth) yet also had characteristics of birds (such as feathers and a wishbone). The implication from such a find is that modern reptiles and birds arose from a common ancestor.

The comparison of similarities between organisms of their form or appearance of parts, called their morphology, has long been a way to classify life into closely related groups. This can be done by comparing the structure of adult organisms in different species or by comparing the patterns of how cells grow, divide and even migrate during an organism's development. Strong evidence for evolution comes from the analysis of homologous structures: structures in different species that no longer perform the same task but which share a similar structure. For examples: the forelimbs of a human, cat, whale, and bat all have strikingly similar bone structures. However, each of these four species' forelimbs performs a different task. The same bones that construct a bat's wings, which are used for flight, also construct a whale's flippers, which are used for swimming. Such a 'design' makes little sense if they are unrelated and uniquely constructed for their particular tasks. The theory of evolution explains these homologous structures: all four animals shared a common ancestor, and each has undergone change over many generations. These changes in structure have produced forelimbs adapted for different tasks. Analogous organs are those which appear similar due to their adaptation for similar functions but are very different in basic structural plan and embryological development. Example: Wings of insects look like the wings of birds and bats as their work is same but the basically different having no bones.

Thus the insect wings are analogous to vertebrate wings and gives evidence of convergent evolution. Stings of honey bee and scorpion perform similar function and look alike. Homology includes a unique group of shared structures referred to as vestigial structures. Vestigial refers to anatomical parts that are of minimal or no use to the organism that possesses them. These apparently illogical structures are

remnants of organs that played an important role in ancestral forms. Such is the case in whales, which have small vestigial bones that appear to be remnants of the leg bones of their ancestors which walked on land. Humans also have vestigial structures, including the ear muscles, the wisdom teeth, the appendix, the tail bone, body hair and the semilunar fold in the corner of the eye.

Taxonomy is the branch of biology that names and classifies all living things. Scientists use morphological and genetic similarities to assist them in categorizing life forms based on ancestral relationships. For example, orangutans, gorillas, chimpanzees, and humans all belong to the same taxonomic grouping referred to as a family called Hominidae. These animals are grouped together because of similarities in morphology that come from common ancestry (called homology).

Embryos of different vertebrates look alike in their early stages, giving the superficial appearance of relationship. In this way the embryos of two or more species provides evidence for a shared ancestor. As the embryo develops, these homologies can be lost and the structures can take on different functions. The tadpole larvae of amphibians bear more resemblance to the young's of fishes than to their own adults. This indicates origin of amphibians from fishes. Similarly, in all birds and mammals including man the embryos pass through stages resembling those of the embryos of fishes, amphibians and reptiles before finally attaining the characters of their respective classes. This proves that all vertebrates have evolved from common fish like ancestors, and also that both birds and mammals have evolved from reptiles.

Every living organism (with the possible exception of RNA viruses) contains molecules of DNA, which carries genetic information. Genes are the pieces of DNA that carry this information, and they influence the properties of an organism. Genes determine an individual's general appearance and to some extent their behavior. If two organisms are closely related, their DNA will be very similar Similarities in DNA are used to determine the relationships between species in much the same manner as they are used to show relationships between individuals. For example: comparing chimpanzees with gorillas and humans shows that there is as much as a 96 percent similarity between the DNA of humans and chimps. Comparisons of DNA indicate that humans and chimpanzees are more closely related to each other than either species is to gorillas.

The formation of complex organisms through 'gradual change' from simple ancestral type over the course of geological time is termed Evolution or Organic. The characteristics of organisms had been changing in the past; they are changing even today, and will continue to do so in the future as well. This is due to the fact that the environment in which organisms live also changes and organisms need to adapt to the changed environment in order to survive. Several living organisms of the past have become extinct. The origin of the various forms (species) found on earth has been a gradual and extremely slow process, requiring hundreds or even thousands of years. However, the evolution of black peppered moth or polyploidy varieties of some crops or pesticide resistant mosquitoes happened in much shorter

period of life. This process of slow and gradual change is called Organic evolution. The theory of organic evolution states that "All living things on earth are here as a result of descent, with modifications from a common ancestor.

Lamarckism (or Lamarckian inheritance) is the idea that an organism can pass on characteristics that it acquired during its lifetime to its offspring (also known as heritability of acquired characteristics or soft inheritance or theory of use and disuse of organs). It was given by the French biologist J. B. Lamarck (1744–1829) who had also coined the terms 'Biology' and 'Invertebrate'. This theory was published in 1809 in his book 'Philosophie Zoologique'.

Lamarck incorporated two ideas into his theory of evolution

- Use and disuse
- Inheritance of acquired traits

Thus Lamarckism can be summarized into four basic points:

- Organisms and their organs have a natural tendency to continuously increase in size generation after generation.
- Continuously changes in environmental conditions directly influence the way of living habits and nature of the organism.
- Growth of less use parts declines while that of better used parts progress.
- Characteristic of poorer growth of some parts and better growth of others, acquired during its lifetime by an organism are hereditary.

Hence these are transmitted to the offsprings for example: Giraffes stretching their necks to reach leaves high in trees (especially Acacias), strengthen and gradually lengthen their necks. These giraffes have offspring with slightly longer necks (also known as soft inheritance).

Darwin's theory of natural selection (Darwinism) is based on the three observations:

- Over production or prodigality of nature (organisms increase in geometric proportion).
- Constancy of number (size of the population remains more or less constant).
- Occurrence of variations (living organisms exhibit variations).

Thus Darwin incorporated two ideas into his theory of evolution:

- Struggle for existence
- Survival of the fittest or Natural selection

Despite overproduction in nature, 'The population size remains more or less constant or steady over the period of time'. This is called constancy of size. The size of the population is determined by various environmental factors like food, shelter, light etc. The struggle for existence is 'the competition that the organism has to face in order to survive'. Due to overproduction, large number of young ones is produced. Not all of them survive because of severe competition for basic

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needs like food, shelter, space, light, water as well as avoid predation, parasitism and diseases. There are three types of struggles namely Intraspecific struggle, interspecific struggle and Environmental struggle.

Variation is 'the difference in characters between the individuals of common descent'. According to Darwin, no two members of any species are exactly alike. He observed changes in characters among the young ones of the same parents. They showed variations with regard to size, color, health, strength etc. These variations are very evident when we observe a group of children of same parents. If this is the case with progeny of single parents, much more are the variations among the members of a population. Thus, variation is an invariable rule in nature. There are three types of variations: useful, harmful and neutral variations. The variations can also be classified as environmental variations and genetic variations. The genetic variations are the variations in the genetic makeup of the individual. They are permanent changes and are heritable. They play an important role in natural selection. In a population 'organisms that possess favorable variations succeed in the struggle for existence and are called the fittest'. It is a type of selection exercised by nature, for example, in a population where there is over production, there is struggle for existence. In this struggle those with more adaptive characters will survive while others will die even before maturity. Hence, the next generation will arise from the best-adapted ones. They will also inherit the characters to the next generation while organisms with unfavorable variations will be eventually reduced and become extinct.

The modern 'synthetic theory' of evolution is the result of important work done by a number of workers in the last few decades. As a result, it was realized that mutations and natural selection both are important for organic evolution. The cause of incompleteness of Darwin's Natural selection theory and De Vries mutation theory was due to the basic principles of genetics discovered in the 20th century were not known to them. Some of the workers who have contributed to the modern synthetic theory are R.S. Fisher, J. B. S. Haldane, Sewall Wright, Mayr and G. L. Stebbins. The modern synthetic theory of evolution involves five basic processes: gene mutation, gene recombination, heredity, natural selection and isolation. Chromosomal mutation such as deletion, duplication, inversion, and translocation also result in variation. The first three factors are responsible for providing genetic variability, while the last two factors are responsible for giving a direction to the evolutionary process. Besides the five factors outlined above, there are two necessary processes, namely migration of individuals from one population to another and hybridization between species and even related genera. These processes increase the genetic variability available to the populations undergoing the process of evolution.

12.2.1 Lamarckism or Lamarckian Postulates

This theory of evolution was published in the book 'Philosphic Zoologique' in 1809 by French scientist Lamarck, Jean Baptiste Pierce Antoine De Monet

Lamarck. His theory was based on the study of fossils of the organisms of the past with their present relatives including the homologous, analogous and vestigial organs. He believed that an organ or structure got modified in the current animal from its ancestor due to the change in the environment and the utility of that particular organ. His theory also said that the organs or structures modified themselves to suit the new needs of the organism.

This theory is popularly known as the inheritance of acquired characters in organisms contained four postulates:

- The internal forces of life tend to increase the size of an organism, i.e., the whole body and also the different parts up to a limit determined by life itself.
- The formation of a new organ or a part in the body is the result of a new need, which has arisen and continues to be felt by the organism.
- The development of an organ and its power of action is directly proportional to its use, continuous and constant use strengthening the organ, while disuse results in its degeneration. This was based on the observation that structures which are subjected to constant use become well developed, where as those are not used tend to degenerate.
- All the changes, which organisms acquire during their life time are transmitted to their offsprings by the process of inheritance. It means changes are cumulative over a period of time. This fourth principle, that is acquired characters are inherited is the most attractive and controversial law, now known as Lamarckian doctrine.

Analysis of Lamarck Theory

The Lamarckism can be summarized into four basic laws:

First Law: This law of Lamarck is merely the growth process of the organism. The increase in size in living beings is common metabolic activities, which are controlled by vital forces of life.

Second Law: This law of development of an organ due to its need is unacceptable to the modern evolutionists. Lamarck thought that change of habits may bring about the modification of existing organs or may initiate the formation of new organ Nobody can believe that any organ can develop, if felt the need of its presence. It means that by simply wanting eye on head, they would develop.

Third Law: This principle of use and disuse of organs were based on direct observation of nature. The continuous and constant use of organs make them efficient and lead to their better development, while disuse for a long time make them undeveloped and ultimately lead to their degeneration. This idea was based on the observation that structures which are subjected to constant use become well developed; where as those those are not used tend to degenerate. This in itself is not an unreasonable proposition. After all everyone knows that exercise and training have the effect on the development of muscles in a weightlifter. Some of the better examples are as follows:

- Ancestors of modern horse lived on soft ground in Jungles. These were browsers. Their foot was plantigrade. These jungles were later replaced by dry grassy plains and these had to graze on hard grass and to work on dry land. These changes in habit were followed by changes in premolars and molars, reduction in number of digits and lengthening of legs. Thus, the foot was gradually changed to unguligrade suited for swift running over hard ground.
- The continuous stretching of neck for several generations by giraffe catch high located leaves and fruits of desert tree caused the lengthening of its neck. This was due to continuous use of particular organ (neck) that enabled him to catch the food (Refer Figure 12.1).

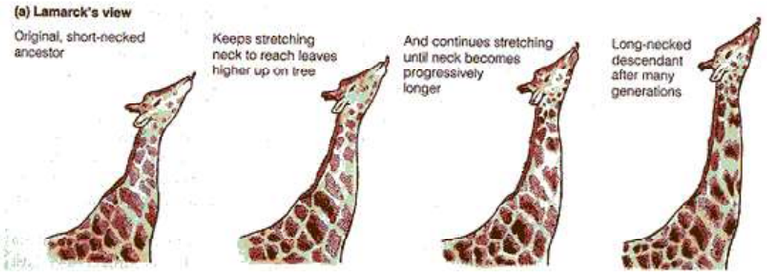

Fig.12. 1 *Elongation of Neck in Giraffe by Continuous Stretching to Catch High Located Leaves*

- Snakes have elongated body accompanied by loss of limbs. The continuous creeping through holes and crevices made limb continuously useless for locomotion with the result that limbs become completely lost in snakes. Their ancestors possess short limbs.
- The flat fishes (deep sea fish) present at the bottom of sea where there no sunlight, led an inactive life, lying on one side of the body. The eye of that side (lying towards bottom) migrated towards upper side and thus, both eyes are on one side of the body. Their larvae possess eyes on either side of the head.
- Besides there are other effects like reduced eyes in moles, vestigial organs in living animals due to disuses; claw in Carnivora; sensitive skin and tactile points on the ventral side of body; callosities of palm in hard workers; webbed feet in swimming birds, etc. exemplifying Lamarckian theory.

Fourth Law: The transmission of acquired characters is the most important usually termed as the Lamarckian doctrine. These acquired characters are hereditary or not, i.e., whether any structural change induced in body by the change in use or disuse or by a change in surrounding environment can affect the germplasm in such a way that offspring will acquire these structural modifications or not, is debatable. Lamarck thought that the favorable structural changes gained by an individual due to use or disuse during its lifetime is preserved and are passed on to its offsprings. In other words, evolutionary change could be achieved by the transmission of

acquired characters. This means that a weight-lifter who has developed enormous arm muscles produces children who are born with especially large arm muscles. These changes become more and more pronounced if exposed to similar stress of the environment as was faced by their ancestors or parents. Likewise in giraffe stretching their neck to reach leaves high in tress, strengthen and gradually lengthen their neck. Theses giraffes have offsprings with slightly longer neck (known as soft inheritance). Such cumulative effects will finally result in the appearance new species (Refer Figure 11.2).

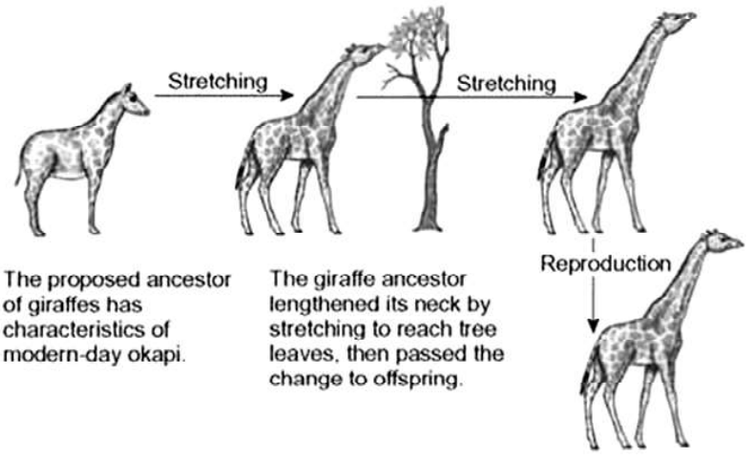

Fig. 12.2 *Evolution and Development of Long Neck Giraffe by Inheritance of Acquired Characters*

Factors Affecting Lamarckian Postulates

A more precise account of Lamarck's theory may be given by the following factors, which played an essential role in evolution:

- **Role of Environmental Factors:** Lamarck believed that various factors like soil, food, temperature causing changes in environment act directly in case of plants, while indirectly in case of animals (since they possess nervous system). The environmental influence leads to change in their habits which results in unusual activity of an organ or structure. In case of animals, Lamarck demonstrated that moles living under ground in dark environment have reduced eyes; fish in deep sea have asymmetric distribution of eyes on head. In case of plants which grown in poor soil remained small and weak and in rich soil they become luxuriant. The leaves of beech tree present on the sunny side have two layers of palisade cells, whereas leaves which present a shade have only one layer. Lamarck, thus, assumed that living organisms react to external conditions and become modified.
- **Competition:** There is a sort of competition in nature not to overcrowd the earth. The stronger organisms try to destroy the weaker ones. The smaller faster, while larger slowly thus, a balance is maintained.

- **Cross Breeding:** These various peculiarities produced in the organisms will always appear in successive generations (provided breeding is confined to such unions only). As a result, crosses between individuals not acquiring these peculiarities result in the disappearance of such characters acquired in particular circumstances.
- **Isolation:** The separation of various generations brings about their different features which in the course of evolution, become specialized into particular species.

Merits and Demerits of Lamarckism

Following are the merits and demerits of Lamarckism

Merits of Lamarckism

Lamarckian theory was simple and provided a way in which change in organism could come about. It was the first completely widespread theory that was offered. It was the theory that lent itself to predictions and therefore to testing. Lamarckian theory proposed that the environment of an organism affected the usage of its various parts. Most persons know that exercise results in larger muscles. In this way, Lamarck remains an important figure in the evolution of evolutionary biology.

Demerits of Lamarckism

There are so many demerits of Lamarckism which are as follows:

- Lamarck suggests the tendency to increase in size but evolution proceeded not only without any increase in size but rather through a reduction in size.
- The second Lamarckian principle that new organs result from new needs is quite false. In other words desire of the animal leads to the formation of new structures not true because it means that the man who mused 'Birds can fly, so why cannot I? Should have grow wings and taken to the air.
- The other Lamarckian principle that the inheritance of characters acquired during the lifetime of the individual found totally incorrect. Many biologists who have devised many types of experiments which have refused it.

Criticism of Lamarckism

This Lamarckism doctrine has criticized very much. Cuvier and Weismann were the great critics of Lamarckism. The following are the main objections against the inheritance of acquired characters:

- **Mutilation Experiment by Weismann:** Weismann was the main opponent of Lamarckian theory. He experimented on white mice by cutting their tails (multilated) but their offsprings did not show any reduction of tails. He obtained 901 youngs from five successive generations of mutilated parents, but none the offspring showed vestigial tail. It means that acquired character (cut tail) was not inherited. Weismann differentiated between changes occurring in the soma a changes occurring in the germplasm. He established

that somatic changes acquired during life time of the organism are non-heritable, where as the changes occurred in the germplasm are inherited by the offsprings.

- **Experiments by Loeb:** Loeb produced artificial parthenogenesis in the sea urchin's egg with the help of chemical stimuli. Similar eggs were produced in corresponding environments in brine shrimp *Artemia salina*, but none of their general showed acquired characters.
- **Experiment by Pavlov:** Pavlov was a Russian scientist, who wanted to show the inheritance learning. He trained the mice to come for food on hearing a bell. But it found that this training was necessary for each and every generation. This clearly proved that acquired characters are not inherited.
- **Experiment by Castle and Phillips:** Recently Castle and Phillips performed transplantation experiments to show that the environment has no effect on heredity. They took a black female guinea pig, its ovary was removed and transplanted into the body of white female pig. The white female (with the ovary from black female) was mated with a white male guinea pig. He found that all the individuals from this pair were black. This clearly showed that environment has no effect on heredity. Hence, this again disapproves Lamarckism. However, some later workers have found that in certain cases environment has some effect on heredity.
- **Traditional Experiment:** Boring of ears and nostrils in Indians has been continued from century among human beings, but their offsprings do not show any trace of holes in ears and nostrils. Females in China wear iron shoes in order to reduce their feet to a short size but their young ones show same normal feet when born.

Likewise, there are numerous cases of non-hereditary nature of acquired characters. Hence may say that variations due to mutilations and somatoplasm based diseases are not inherited: otherwise, none of us would exist without some trace of hereditary crippling. Thus, this inheritance may prove disastrous for the well-being of human population by inheritance of undesirable acquired characters.

12.2.2 Neo-Lamarckism

Besides the above, there are evidences which support that acquired characters are inherited from generation to generation. The categories of evolutionists who support the Lamarckian doctrine of inheritance of acquired characters come under the heading of Neo-Lamarckians. Among the Neo-Lamarckians, notable supporters are Herbert Spencer, Eimer, Cope, Hyatt, Dall, Packard, Nagaeli, Haeckel and Gadow. These workers have suggested that if not all some of the acquired characters are inherited to some extent. The following are the evidences of transmission of acquired

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Characteristics of Non-Lamarckism

- **Experiment by Sumner:** BF.B. Sumner reared the white mice at 20°C to 30°C. He found that due to this high temperature they develop longer bodies, long tail and longer hind feet. This abnormality was found to be transmitted to the offsprings generation after generation.
- **Experiment by Lindsey:** Lindsey subjected the cold-blooded animals, warm-blooded animals and plants to unusual environmental conditions. He saw that changes due to these conditions are transmitted to some extent in their offsprings.
- **Experiment by Brown Sequard:** Various acquired diseases by Brown Sequard are inherited from generation to generation. For example: Exophthalmia was caused in parents by injuring the restiform body (in brain) with the result of protrusion of eyeballs. This disease was later on inherited to the later generations. The other disease like Haematoma and dry gangrene are produced (ear alternations) by injury to the restiform body near the rib of calamus. Later these were found to be inherited.
- **Experiment by McDougall:** McDougall trained white rats to escape from a tank of water by certain route. These trained rats were mated and their offsprings in turn were thought the problem of escape. This was repeated for 44 generations. He found there was a marked and progressive decrease in the number of errors by white rats.
- **Experiment by Guver and Smith:** Guver and Smith induced the hereditary changes in the eyes of fetuses of rabbit by simply destroying lens of living female with a needle in situ. The anti-lens serum has been produced in the blood of these animals. The effects seem to be inherited in their generations.
- **Experiments by Griffith and Detlefson:** Griffith and Detlefson recently performed the interesting experiments to prove the inheritance of acquired characters. They reared rats in cages placed on rotating table for several months. Consequently, they become adapted to the rotating condition to such an extent that, when rotation was stopped, they showed signs of nystagmus (dizziness) and other conditions. This condition was inherited for several generations.
- **Experiment by Kammerer:** He worked on *Proteus anguinus* (amphibian) living in underground cave's water in complete darkness. It was blind (vestigial eye covered over by skin) and colorless. He brought *Proteus* into daylight, due to which it become colored (brown and black), which passed on to its progeny. Eyes also developed normally in day light: This shows that changed environments have induced the changes in the animals and the acquired characters were inherited.

The above objections and evidences on the inheritance of acquired characters are most unconvincing and unsatisfactory. The main theories in his regard are

'mneme' theory of Semon and 'centro-epigenesis' theory of Rignano. Semon holds that every condition of life or functional activity of organism leaves a permanent record of itself in the form of 'engramme'. If conditions are continued for long period, these engrammes affect heredity and become inheritable, but if they are of short duration they do not inherit. Now it is clearly established that only those characters, which in affect germplasm (germinal or blastogenic) can be inherited, while other characters (somatic or somatogenic) affecting body cells do not transmit to their offsprings.

12.2.3 Darwinian Theory or Darwinism

Charles Darwin was born (12 February, 1809) in England sailed on H.M.S. Beagle for world exploration and visited some of islands of Atlantic ocean, coast of South America and some islands of South Pacific. During his voyage he noted the flora and fauna of many islands and collected numerous living and fossil specimens. Beagle also later sailed to the Galapagos islands on the west coast of America. He observed great variations among the organisms that lived on these islands and exhibited radiations and modifications in form. He found giant tortoises distinctly different on each island. The common birds, finches were markedly different from the finches of main land. A.R. Wallace and C. Darwin both read Malthus's views on population, which state that population increases in a geometrical ratio, whereas food in arithmetical ratio. In 1859, these views were incorporated in the book, Origin of Species by Natural Selection or the preservation of favored races in the struggle for life. The great scientist C. Darwin was died on 19 April, 1882.

The idea of natural selection is very simple, though its operation is highly complex and may be extremely subtle. Darwinism explains how evolution might have occurred in nature. Darwinism is based on three facts of nature can be summarized as follows:

- Organisms multiply in geometric ratio (Over production).
- Over production and number of survivors remains roughly constant (Struggle for existence).
- Struggle for existence and variations and heredity (Survival of the fittest and natural selection).
- Survival of the fittest and continued changes or adaptations in the organisms (Origin of new species).

Over Production (Prodigality or Fecundity)

According to Darwin, the driving force is provided by the tendency of living beings to increase their numbers rapidly. For examples, Fishes are noted for laying large numbers of eggs about 1,700,000 eggs. The similar prodigality of the salmon in egg production is common. One female toad may lay as many as 12,000 eggs. It has been calculated that one pair of houseflies breeding in April would have by August, if all eggs hatched and all resulting individuals lived to reproduce in their

turn, $1.9x10^{20}$ descendants. According to Darwin the elephant is the slowest breeder of all known animals and have longer intervals between generations. It begins breeding when thirty years old, and goes on breeding till ninety years old, producing six youngs in the interval and each surviving till one hundred years old. If this be so, after a period of from 740-750 years there would be nineteen million elephants alive descended from the first pair. Why, in fact, do we not find our lakes choked so with fish, our fields carpeted with toads, the earth overrun with elephants and so on? Because, for each species, there are certain checks or limiting factors, that opposes such an increase in number. The most important of these checks are limited food supply, predatory animals, diseases, space restrictions and inanimate environment.

Struggle for Existence

It has been noticed that in each generation each species attempts to produce many more individuals that can hope to live to maturity under the prevailing limited factors. The result is a competition among the offspring for food, mates, home territories and strive to survive the aggressions of predatory animals, disease, and the severities of inanimate nature. Darwin called this process 'the struggle for existence'. In this struggle those will succeed that have favorable or advantageous inheritable variations of structure, physiology and so on. Those individuals will fail that have no such variations or that have unfavorable or harmful variations. It is true that non inheritable and favorable variations will not make an individual to survive. Therefore such variations have no 'future' as regards improvement of the species concerned. The inheritable variations arise as new mutations and as new combination of genes originating in various ways. Darwin himself placed great stress on the importance of variations, including individual differences, and he recognized that they must be inheritable for the usefulness in evolution. He also knew that variation is universal, that 'No Two Individuals Are Alike'.

- **Success in the Struggle for Existence:** Darwin laid more stress upon survival. Individuals having favorable inheritable variations survive, while their less highly endowed individuals die. This phenomenon has been termed "the survival of the fittest. The 'fittest were thought of as those individuals that possess inheritable characteristics enabling them to succeed in the 'struggle for existence' in the particular circumstances and environment in which they live. Since they are the survivors, the fittest then become the parents of the next generation, whose members inherit the favorable characteristics from their parents. The most successful individuals or groups that contribute their genes in greatest number to the generation. So far as contribution to evolution is concerned, a living animal that does not reproduce is supposed to be dead, because he consumes food without making any contribution to the species in return. But individuals that live together in societies and do not reproduce, but provide essential services to the society of which they are a part, they also contribute their services to the next generation. Thus, be it on the individual or on the social level, success in the

'struggle for existence' means success in contributing to the next generation. Its examples are social insects as ants and bees.

- **Nature of the Struggle for Existence:** So far we have stressed competition between individuals in the same species for food, territory, etc., this is intraspecific competition. Interspecific competition also occurs and at times it is important evolution. Two closely related species (recently arisen from a common ancestral species, perhaps) may compete for the same food supply. If this competition is keen it may lead to changes in the two species so that competition will be lessened. Thus, two species of ground finch living on the same Galapagos island may come to differ from each other in beak size, one specializes to feed on large seeds and the other on small seeds. In this case, one species may be so much more efficient than other in utilizing food supply that the less efficient species becomes extinct, at least in the territory originally shared by both.

The resistance to disease is highly important; ability to produce large numbers of viable offsprings is advantageous particularly in those species in which parents do not care for the young after hatching or birth; youngs that develop quickly have an advantage over those that slowly. When two competing strains differ in speed of that strain which produces mature offspring in less time will contribute more of its genes to further generations than that in which sexual maturity attained more slowly. Thus natural selection results from the cumulative action of all forces tending to ensure that individuals possessing one genetic constitution shall leave larger number of offspring than those individuals possessing some other constitution.

Variation Under Nature

The fact that no two organisms or parts of the organisms are exactly alike, no matter how closely related, is a commonly observed phenomenon. These differences are called variations. It is the basic prerequisite and progressive factor for evolution, because without variations, no change could occur and evolution would be impossible. But all the variations are not significant from evolutionary point of view. Some of them are changes occurred temporarily in the soma of the organisms and are not inherited to the offsprings. Only those variations which can be inherited can take part in the evolution of species. These variations are called heritable variations. Changes occurred in the genes, or the chromosomes of the germplasm are the only heritable variations. Some of the visible variation results from environment influences, particularly diet, and this play no direct part in the evolutionary process. Darwin observed that the various useful variations are selected by individuals and thus, evolution results. Darwin assumed variations as axioomatic without describing their real nature and origin, in plant and animals.

Survival of the Fittest

Due to these various struggles for existence and useful heritable variations, only those individuals survive, which are best fitted to new conditions of life and the least fit are the first to perish. The well adapted individuals reach reproductive age

and hand over their favorable characteristics to their offsprings, whereas less well-adapted individuals fail to do so. Nature select those individuals, which are sufficiently well-adapted and allows them to survive, and rejects those that are poorly adapted. The later usually perish before they reach sexual maturity. Darwin coined the term natural selection to describe this weeding out process. As environmental conditions are constantly changing, natural selection is forever favoring the emergence of new forms. The survival of the fittest is the result of natural selection which enforces adaptations

12.2.4 Origin of Species

One mutation in a population causes usually a small change. Addition of one mutation to another probably accounts for many of the larger differences distinguishing separate species, genera and so on. In later generations a second mutation arises that is an improvement on, or addition to the first one. Natural selection now works on this second mutation until some generations later the whole population comes to possess it. Thus, step by step through the long expanses of geologic time greater and greater evolutionary change is produced by natural selection. The change as we have described will be in the nature of more perfect adaptation to the environment in which the animals are living, i.e., postadaptation. (More and more perfect adaptation to a stable environment in which species is already living. This type of adaptation is called postadaptation, since species has already entered the environment, and additional adaptation only perfects the animal for living under the prevailing conditions).

Suppose that the environment in which our hypothetical population live changes. This might result from geologic change in the inhabited region from climatic change, from biotic change or from that a portion of the population migrates into different region from the former region. As a result different mutations will prove advantageous in the struggle for existence, and the result will be that the population or a portion of it, gradually differ from the ancestral population. Eventually a descendent population may come to differ so much from the ancestral one that a biologist concludes that the two should be regarded as separate species An ancestral population may give rise to two or more descendant populations. If these "sister" populations become adapted to different environments they may in the course of time become sufficiently different from each other as to be considered separate races. If change continues they eventually become separate species

Criticism (Objections) to Darwinism

Some of the objections to the theory of natural selection, which Darwin explained vaguely, are the following:

- If species have descended as a result of gradations, there should be innumerable transitional stages and the species should not be so well defined as we see them to be. Darwin's theory stresses upon small fluctuating variations, which are to a large extent nonheritable and can play no part in the evolution.

- How can natural selection bring about characters of no use like the tail of Giraffe of trifling importance? It does not explain the effect of use and disuse and presence of vestigial organs.
- It could not explain whether the instincts are acquired and modified through natural selection or not.
- He did not differentiate between somatic and germinal variations and considered all variations as heritable.
- How some species, when crossed, produce sterile offsprings, whereas when varieties are crossed they produce fertile offsprings?
- Darwin described the survival of the fittest, but not the arrival of the fittest.
- Over-specialization (some organs began to develop enormously in relation ton to size of body causing harm to individuals). For example, antlers of extinct Irish deer cause its extinction.
- Degeneration of organs.
- Darwin proposed artificial selection for improving races of domestic plants and animals, but these could never lead to definite or permanent specific variations.
- Natural selection does not explain the evolution of land animals from aquatic ones.
- It appears somewhat absurd that variations tending in an infinitesimal degree should be preserved.
- Darwin's sexual theory involves passively on the part of male and an active choice on the part of female for a more beautiful, attractive and more powerful male. This theory was most criticized.

Neo-Darwinism

Since Darwin's natural selection theory, there have been many researches and profound changes and likewise the theory has been modified. These all supporters constitute Neo-Darwinians including Wallace, Huxley, Ernst Heinrich, Haeckel, August Weismann, Mendel, DeVries, etc. The following are the main recent experiments put forward to support the natural selection theory:

Weldon's Experiments on Shore Crab

Weldon experimented with the shore crabs of Plymouth Sound, which showed that, as a result of changed climatic conditions, natural selection produces an alteration in species by acting on minute variations as Darwin held. During demonstration of these experiments, the rate of flow of river water was slowed down by placing a large break in water near the mouth of Plymouth Sound. With this slowing down of flow, fine China-clay sediment settled more, resulting in the death of numerous crabs of species *Carcinus maenas*. The survivors had a slightly narrow frontum and there was a progressive narrowing of the frontum in succeeding

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generations. This shows that under changed environmental conditions natural selection operates upon minute fluctuating variations.

Cesnola's Experiments with Mantis

Cesnola tested the selective value of various colors of Mantis by fixing them on plants. Those colors which were harmonious with plants escaped, while others of different colors were eaten up by birds.

Poulton's and Sander's Experiments with ButterflyPupae

Many butterfly pupae of different color patterns were placed under the conditions favoring protective colors. Some of them were also kept in nonhormonious background. They concluded that protective coloration is a real survival factor.

Davenport's Experiments with Chickens

A larger number of variously tinted chickens, for example, black, gray while, barred etc. were allowed to wander free in fields. Most of them which were white and easily distinguishable were killed by enemies like hawks, kites etc., while others escaped due to conformity of colors with surroundings.

Check Your Progress

1. List the constant modifications that affect basic concept of organic evolution.
2. What were the two ideas incorporated by Lamarck in his theory of evolution and how it can be summarized?
3. What are the factors in which Darwin's theory of natural selection based?
4. What are the two ideas of Darwin that he incorporated his theory of evolution.
5. State Lamarck's first law.

12.3 THEORY OF NATURAL SELECTION

Darwin's Natural selection theory was the first milestone in the evolutionary history in which detailed mechanism about origin of species has been discussed. After publication of the book entitled '***Origin of Species***' by Darwin in 1859, major scientific progress has been made in the field of evolutionary biology elucidating the roles of various factors operating in Nature and responsible for the origin of species. Darwin had no knowledge about the scientific basis of these factors such as selection variations, isolation and mutations which operate in Nature and all collectively lead to the speciation and species formation is now grouped under Modern Synthetic Theory of Evolution, (Refer Figure 12.3). The modern analysis of old postulates of Darwin's Natural Selection theory has brought forward new scientific data which have proved to be quite valuable in better understanding the mechanisms of origin of species at genetic and molecular level. Some of these modified factors are given below.

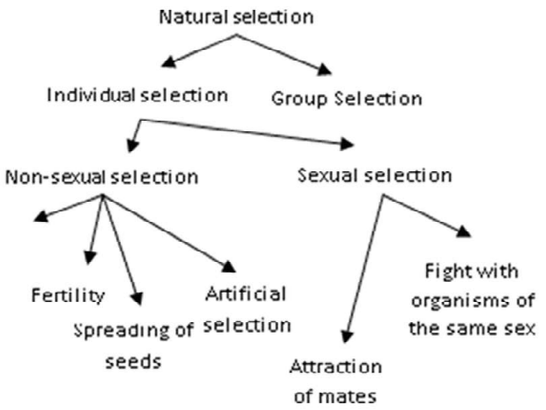

Fig. 12.3 *Theory of Natural Selection*

Differential Reproduction

Darwin emphasized the phenomena of overproduction in living organisms in nature. However, he could not visualize the genetic basis of reproduction because of lack of scientific knowledge at that time. In Nature, differential reproduction starts with the interaction between environment and phenotypes and terminates in increased reproductive and survival value of the species. Differential reproduction (recombination) of origin of species causes changes in the gene frequency from one generation to other and finally results in gene pool variations. It is achieved either by increased rate of reproduction or by reducing vulnerability to environmental changes which cause mortality. Thus, natural selection begins with differential reproduction of genotypes in a population (gene pool).

Retention of Favorable Characteristics

Natural selection favors those organisms which possess high selective value for these beneficial and favorable changes. It encourages those genes which ensure great degree of adaptive efficiency between population and environment. It is well exemplified in a population of fruitfly, *Drosophila melanogaster*. It was observed that in the population, all the females (either red or white-eyed) prefer to mate with wild red-eyed males. During this process, white-eyed gene (trait) is eliminated from the due to natural selection via differential mating. Thus, natural selection favors the efficient gene contribution and retention of those characteristics, in a gene pool which is exposed to various factors of natural selection.

Occurrence of Inheritable Variations

Darwin considered minute fluctuating variations as the principal factor for natural selection. However, they are mostly non-heritable. In the light of modern researches, it has been observed that chromosomal variations and gene mutations are inheritable and are responsible for the variations in the organisms of a population. It is the genotype which changes due to influence of the environment. Natural selection

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favors only suitable (adaptive) variations and less-adapted variations are lost from the population with the death of organisms. These adaptive variations are accumulated in the gene pool of population and lead to speciation.

Selection

Darwin gave an idea of artificial and sexual selection mechanism in nature. His findings were, however, not based on scientific data and thus, unscientific. Selection is one of the most important prerequisite for species formation. Various kinds of selection are detailed below:

- **Stabilizing (Normalizing) Selection:** It maintains the equilibrium of gene pool, brings out homogeneity and weeds out continually the regenerated and less fit or more specialized genotypes in sexually reproducing populations. During this process, natural selection acts over the organisms in the absence of large scale environmental changes for long periods, keeping the population genetically constant by favoring normal genotypes and elimination extremely variable phenotypes. Elimination may be artificial or genetic death. Stabilizing selection can be well illustrated with examples of land snails and sparrows. Weldon experimented on land snails and observed that only those snails, survived for longer periods, whose shell whorls, were normal. On the other hand, abnormal land snails with inner whorl of shell (irregular) were gradually eliminated. Therefore, stabilizing selection leads to reduction in the variation and making population more homogeneous.
- **Progressive or Directional Selection:** It produces a regular change within a population along certain directions with respect to specific traits. During this, environmental changes occur slowly and progressively along certain directions. The directional selection favors non-average or extreme phenotypes and then pushes these phenotypes of a population in the direction of the average phenotypes. Therefore, it indicates progressive selection which removes individuals from one end of the normal and adds towards the other end. This type of selection takes place during or after environmental change. The gene frequency of a particular trait (gene) changes more or less in one direction over long periods. Best example of progressive selection in nature is shown by peppered moth, *Biston bitularia* found in Europe. It had mottled greenish grey color of body. In 19th century, there happened great industrialization in Europe which resulted in the production of black smoke. This black smoke got deposited on trees and changed the color of trees and insects, i.e., mottled grey color to black color. To escape from enemies, these moths developed adaptive pigmentation which made them survived.
- **Disruptive Selection:** It refers to splitting or break-up of previously homogeneous population into several different adaptive forms. It pushes the phenotypes within a population away from the population average by supporting the values at the two ends of variability curve. It happens when

a population previously adapted to heterogeneous environment is subjected to divergent selection pressures in different parts of its distributional area. It maintains balanced polymorphism in the population based on frequency dependent adjustments to heterogeneous environment and tends to increase the amount of variance within a population. Stebbins and co-workers studied the disruptive selection in a population of sunflowers plants in California over a period of 12 years.

Mutations

The modern synthetic theory in a nut shell maintains that mutation and sexual recombination furnish the raw materials. The natural selection fashions from these materials genotypes and gene pools. And in sexually reproducing forms, the arrays of adaptively coherent genotypes are protected from disintegration by reproductive isolating mechanisms. Modern synthetic theory of evolution is a combination of mutations, variations, heredity, isolation and natural selection. Alteration in the chemistry of gene (DNA molecule) is able to change its effect and is called gene mutation. Mutations can produce drastic changes or can remain insignificant. There are equal chances of a gene to mutate back to normal. Most of the mutations are harmful or deleterious but not all. Most of the mutants are recessive to normal gene and these are able to express only in homozygous state. Thus, the mutations produce variations in the offsprings.

Natural Selection

The organisms which are more suited for environmental conditions will survive. They will overpower the forces of competition. Organisms with suitable genotype have better opportunities of survival and reproduction, contributing more offsprings to the population. However, those with less suitable characteristics and genotypes contribute less offsprings to the population and gradually decrease in numbers. Thus, it is the genotype which actually changes under the influence of environment, variations caused by changes in the genes (gene mutations) and in the chromosomes (chromosomal mutations) which produce heritable variations which are found suitable, the less suited or harmful variations are lost from the population with the death of the possessors. The variations are accumulated in the gene pool of the population and therefore, the population becomes diverged either from the parent population or from sister populations.

Isolation

Isolation (segregation) of organisms of a species into several populations or groups under psychic, physiological or geographical factors is supposed to be one of the most important factors responsible for evolution. Geographical isolation includes physical barriers like high mountains, rivers, oceans and long distances preventing interbreeding between related organisms. Physiological barriers help in maintaining the individuality of the species, since these isolations do not allow the interbreeding amongst the organisms or different species. It is the reproductive isolation.

Origin of New Species

The populations of a species present in different environments and are segregated by geographical and physiological barriers, accumulate different genetic differences due to mutations, recombination, hybridization, genetic drifts and natural selection. These populations thus, become different from each other morphologically and genetically, and they become reproductively segregated, forming species. Thus, new species arise by the establishment of some reproductive barrier or isolation, which checks the free gene flow among population of different environments.

Variation

Variation, in biology is any difference between cells, individual organisms, or groups of organisms of any species caused either by genetic differences (genotypic variation) or by the effect of environmental factors on the expression of the genetic potentials (phenotypic variation). Variation may be shown in physical appearance, metabolism, fertility, mode of reproduction, behavior, learning and mental ability, and other obvious or measurable characters:

- **Genotypic Variations:** These are caused by differences in number or structure of chromosome or by differences in the genes carried by the chromosomes. Eye color, body form, and disease resistance are genotypic variations. Individuals with multiple sets of chromosomes are called polyploid; many common plants have two or more times the normal number of chromosomes and new species may arise by this type of variation. A variation cannot be identified as genotypic by observation of the organism; breeding experiments must be performed under controlled environmental conditions to determine whether or not the alteration is inheritable.
- **Phenotypic Variation:** Environmentally caused variations may result from one factor or the combined effects of several factors, such as climate, food supply, and actions of other organisms. The phenotypic variations also include stages in an organism's life cycle and seasonal variations in an individual. These variations do not involve any hereditary alteration and in general are not transmitted to future generations; consequently, they are not significant in the process of evolution.
- **Quantitative and Qualitative Variations:** Quantitative variation is also called as continuous variations (smoothly grading between two extremes, with the majority of individuals at the centre, as height in human populations); or as discontinuous, or qualitative (composed of well-defined classes, as blood groups in man). A discontinuous variation with several classes, none of which is very small, is known as a polymorphic. The separation of most higher organisms into males and females and the occurrence of several forms of a butterfly of the same species, each colored to blend with a different vegetation, are examples of polymorphic variation.
- **Genetic Variation:** It means that biological systems (individuals and populations) are different over space. Each gene pool includes various

alleles of genes. The variation occurs both within and among populations, supported by individual carriers of the variant genes. Genetic variation is brought about, fundamentally, by mutation, which is a permanent change in the chemical structure of chromosomes. Genetic recombination also produces changes within alleles. The genetic variation among individuals within a population can be identified at a variety of levels. It is possible to identify genetic variation from observations of phenotypic variation in either quantitative traits (traits that vary continuously and are coded for by many genes (for example, leg length in dogs)) or discrete traits (traits that fall into discrete categories and are coded for by one or a few genes (for example, white, pink, red petal color in certain flowers)).

- **Geographic Variation:** It means genetic differences in populations from different locations. This is caused by natural selection or genetic drift.
- **Variations and Heredity:** The nature of genetic variations caused by reshuffling of genes during sexual reproduction was very little known at the time of Darwin. The phenomenon of meiosis causes random assortment of genes during synapses and rearrangement of maternal and paternal chromosomes in both kinds of gametes. Such a reassortment of genes in a large population with large gene pool is the basis of appearance of new organisms. The crossing over of genes during meiosis also adds to the variations and chromosomal abberations like inversion, translocation and polyploidy also result in the origin of new species. The transmission of characteristics or variation to offsprings, is an important mechanism of evolution. Organisms possessing hereditary characteristics that are helpful, either in the animal's native environment or in some other environment that is open to it, are favored in the struggle for existence. Consequently, the offsprings are able to benefit from the advantageous characteristics of their parents.

Evolution of Races to Species

Interbreeding nature of a population serves as the important cohesive force that holds it together and enables it to share a common gene pool. A species may consist of numerous individual populations with various degree of interbreeding between them. On the other hand widely separated populations will have less opportunity to share their gene pool than those are closer. The structure of a species is therefore broken into various geographical subunits. Since the forces acting upon these subunits may vary may vary in different localities, hence there will be some observable differences between populations.

Adaptive changes in gene frequencies have been found in industrial melanism. Industrial areas in England and Western Europe have seen a marked change in the appearance of certain moths and butterflies during the past century from light-colored forms to dark-colored melanic forms. The genetic basis of these differences

generally involve a single, usually dominant, gene determining melanism, as well as a number of modifier genes that affect the dominance of the melanic gene.

In the main populations of the same species that differ markedly from each other have been characterized as races. The races share participating in the gene pool of the entire species, although they are sufficiently separated to exhibits individually unique gene frequencies. The distinction between races is that they may differ in relative frequency of a particular gene, but these differences do not prohibit gene exchange. Populations of a species showing both phenotypic and genotypic differences in response to climate are called geographic races. Thus, geographic aggregates of populations that differ in genetic traits are called races.

Race formation is accelerated by barriers that reduce gene exchange between populations. Initially such barriers are primarily geographical and occur when populations are separated from one another and occupy different areas. The potential for gene exchange enables all the different populations to be considered as members of a single species. When populations have achieved sufficient differences to inhibit any gene exchange between them and have diverged sufficiently, then they reached at the level of separate species.

Check Your Progress

6. What causes gene pool variations?
7. How is gene pool variations achieved?
8. What is isolation?

12.4 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. The basic concept of organic evolution is continuity of life with constant modifications which takes place due following reason:
   - Environmental conditions in nature are unstable and ever changing.
   - Organisms have an inherent potentiality of changing according to the changing environmental condition called adaptability which leads to evolution of new species.
   - All the present species had common ancestors at some or the other time of their evolutionary history.
2. Lamarck incorporated two ideas into his theory of evolution:
   - Use and disuse
   - Inheritance of acquired traits

Thus Lamarckism can be summarized into four basic points:

- Organisms and their organs have a natural tendency to continuously increase in size generation after generation.
- Continuously changes in environmental conditions directly influence the way of living habits and nature of the organism.
- Growth of less use parts declines while that of better used parts progress.
- Characteristic of poorer growth of some parts and better growth of others, acquired during its lifetime by an organism are hereditary.

3. Darwin's theory of natural selection (Darwinism) is based on the three observations:
   - Over production or prodigality of nature (organisms increase in geometric proportion).
   - Constancy of number (size of the population remains more or less constant).
   - Occurrence of variations (living organisms exhibit variations).
4. Darwin incorporated two ideas into his theory of evolution:
   - Struggle for existence
   - Survival of the fittest or natural selection
5. Lamarck first law states that the growth process of the organism. The increase in size in living beings is common metabolic activities, which are controlled by vital forces of life.
6. Differential reproduction (recombination) of origin of species causes changes in the gene frequency from one generation to other and finally results in gene pool variations.
7. Gene pool variations.is achieved either by increased rate of reproduction or by reducing vulnerability to environmental changes which cause mortality. Thus, natural selection begins with differential reproduction of genotypes in a population (gene pool).
8. Isolation (segregation) of organisms of a species into several populations or groups under psychic, physiological or geographical factors is supposed to be one of the most important factors responsible for evolution.

12.5 SUMMARY

- The term evolution was first used by Herbert Spencer. The evolution has seen defined as a gradual change from one condition to another. In other words, biological evolution is genetic change in a population from one generation to another.

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- According to Darwin, the evolution can be defined as 'Descent with modifications'. Continuous evolution over many generations can result in the development of new varieties and species.
- The evolution in which species adapted to different environmental conditions, diversify and modify along many divergent changes known as divergent evolution. However several species or organisms belonging to various regions and groups migrated in to a common environment or habitat and modified and evolved accordingly.
- The study of Fossils provides evidence about the changes in organisms over long periods of time. Modern paleontology began with the work of Georges Cuvier (1769–1832). Cuvier noted that, in sedimentary rock, each layer contained a specific group of fossils.
- The same bones that construct a bat's wings, which are used for flight, also construct a whale's flippers, which are used for swimming. Such a 'design' makes little sense if they are unrelated and uniquely constructed for their particular tasks.
- The theory of evolution explains these homologous structures: all four animals shared a common ancestor, and each has undergone change over many generations. These changes in structure have produced forelimbs adapted for different tasks.
- Analogous organs are those which appear similar due to their adaptation for similar functions but are very different in basic structural plan and embryological development. Example: Wings of insects look like the wings of birds and bats as their work is same but the basically different having no bones.
- Vestigial refers to anatomical parts that are of minimal or no use to the organism that possesses them. These apparently illogical structures are remnants of organs that played an important role in ancestral forms.
- Taxonomy is the branch of biology that names and classifies all living things. Scientists use morphological and genetic similarities to assist them in categorizing life forms based on ancestral relationships. For example, orangutans, gorillas, chimpanzees, and humans all belong to the same taxonomic grouping referred to as a family called Hominidae.
- Lamarckism (or Lamarckian inheritance) is the idea that an organism can pass on characteristics that it acquired during its lifetime to its offspring (also known as heritability of acquired characteristics or soft inheritance or theory of use and disuse of organs).
- Charles Darwin was born (12 February, 1809) in England sailed on H.M.S. Beagle for world exploration and visited some of islands of Atlantic ocean, coast of South America and some islands of South Pacific. During his voyage he noted the flora and fauna of many islands and collected numerous living

and fossil specimens. Beagle also later sailed to the Galapagos islands on the west coast of America.

- The 'fittest were thought of as those individuals that possess inheritable characteristics enabling them to succeed in the 'struggle for existence" in the particular circumstances and environment in which they live. Since they are the survivors, the fittest then become the parents of the next generation, whose members inherit the favorable characteristics from their parents.
- Darwin observed that the various useful variations are selected by individuals and thus, evolution results. Darwin assumed variations as axioomatic without describing their real nature and origin, in plant and animals.
- Natural selection favors only suitable (adaptive) variations and less-adapted variations are lost from the population with the death of organisms. These adaptive variations are accumulated in the gene pool of population and lead to speciation.
- The modern synthetic theory in a nut shell maintains that mutation and sexual recombination furnish the raw materials. The natural selection fashions from these materials genotypes and gene pools.
- Physiological barriers help in maintaining the individuality of the species, since these isolations do not allow the interbreeding amongst the organisms or different species. It is the reproductive isolation.
- The populations of a species present in different environments and are segregated by geographical and physiological barriers, accumulate different genetic differences due to mutations, recombination, hybridization, genetic drifts and natural selection.
- A species may consist of numerous individual populations with various degree of interbreeding between them. On the other hand widely separated populations will have less opportunity to share their gene pool than those are closer.

12.6 KEY WORDS

- **Evolution:** Evolution is change in the heritable characteristics of biological populations over successive generations.
- **Species:** In biology, a species is the basic unit of classification and a taxonomic rank of an organism, as well as a unit of biodiversity.
- **Reproduction:** Reproduction (or procreation or breeding) is the biological process by which new individual organisms offspring are produced from their parents.
- **Mutation:** In biology, a mutation is the permanent alteration of the nucleotide sequence of the genome of an organism, virus, or extrachromosomal DNA or other genetic elements.

- **Homology:** Homology includes a unique group of shared structures referred to as vestigial structures.
- **Taxonomy:** Taxonomy is the branch of biology that names and classifies all living things.
- **Organic:** The formation of complex organisms through 'gradual change' from simple ancestral type over the course of geological time is termed Evolution or Organic.
- **Variation:** Variation is the difference in characters between the individuals of common descent.

12.7 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short Answer Questions

1. What are the factors that affect Lamarckian postulates?
2. Write a brief account on survival of the fittest.
3. Give short note on origin of species.
4. Brief a note on Cesnola's experiments with Mantis.
5. Write short note on the following:
   - Selection
   - Mutations
   - Natural selection
6. Give a general account on variation.

Long Answer Questions

1. Describe the theory of inheritance of acquired characters.
2. Give an account of Lamarck's theory of organic evolution.
3. Outline the different postulates of Lamarckism and discuss their merits and demerits.
4. Discuss Darwin's theory of 'Natural Selection' in the light of recent studies.
5. Write an essay on Neo-Darwinism.
6. What is the modern concept of organic evolution?

12.8 FURTHER READINGS

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UNIT 13 EVIDENCES FOR EVOLUTION AND ADAPTATION PATTERNS

Structure

13.0 INTRODUCTION

The remains or traces of organisms from a past geologic age embedded in rocks by natural processes are called fossils. They are extremely important for understanding the evolutionary history of life on Earth, as they provide direct evidence of evolution and detailed information on the ancestry of organisms. The Nobel Prize winning scientist Linus Pauling aptly described science as the search for truth. Science does this by continuously comparing its theories objectively with evidence in the natural world. When theories no longer conform to the evidence, they are modified or rejected in favor of new theories that do conform. In other words, science constantly tries to prove its assumptions to be false and rejects implausible explanations. In this way, scientific knowledge and understanding grow over time. There are convincing proofs about the existence of evolutionary mechanisms in living animals and plants. Various facts and observations have been put forward as evidences to the categories, such as, evidences from classification, morphology (comparative anatomy), comparative physiology and biochemistry, cell biology, embryology, paleontology, and geographical distribution.

All animals and plants are alike being composed of protoplasm organized in the form of cells. The various organ systems of vertebrates are constructed on a basic plan and the minor differences visible in some forms are the adaptive modifications to the diverse mode of living reflect common ancestry.

In this unit, you will study about evidences of evolution, species and speciation and isolation and of isolation for speciation in detail.

13.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand the evidences of evolution
- Discuss the species and speciation
- Explain the isolation and of isolation for speciation

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13.2 EVIDENCES OF EVOLUTION

The remains or traces of organisms from a past geologic age embedded in rocks by natural processes are called fossils. They are extremely important for understanding the evolutionary history of life on Earth, as they provide direct evidence of evolution and detailed information on the ancestry of organisms. The Nobel Prize winning scientist Linus Pauling aptly described science as the search for truth. Science does this by continuously comparing its theories objectively with evidence in the natural world. When theories no longer conform to the evidence, they are modified or rejected in favor of new theories that do conform. In other words, science constantly tries to prove its assumptions to be false and rejects implausible explanations. In this way, scientific knowledge and understanding grow over time. Religious explanations for the order of things are not science because they are based primarily on faith and do not subject themselves to be objectively falsified. Because of this fundamental difference in the approach to understanding our natural world, the U.S. Supreme Court in effect decided in 1987 that the Biblically based 'creation science' is not a science and cannot be taught as such in public schools as an alternative or in addition to the mainstream evolutionary theory of the biological sciences. However, religious creation stories and the idea of 'intelligent design' can be taught in philosophy, religion, or history courses. Religion and Science provide different approaches to knowledge. It is important to understand both.

Biologists believed that a species was ideally represented as a particular morphological type distinctly different from the morphological types of other species. According to Darwin, morphological species types arose by 'Natural Selection' among the variable members of previous species. Population numbers increase by normal methods of reproduction. Most populations were restricted by their environment to a stationary size. Darwin called it Struggle for Existence. This struggle was not necessarily a battle between organisms. It was death that occurs by any means in preventing all off springs of a species from surviving to reproductive age. Struggle for existence consequently led to the selection and proliferation of only those organisms that were adapted to their environment and those most successful in mating. The result of selection was described as 'Survival of the Fittest' a term borrowed from Herbert Spencer. In modern terms,' fittest' means only individuals with most reproductive success in a particular environment, whether such success is achieved by struggle or weakness.

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There are convincing proofs about the existence of evolutionary mechanisms in living animals and plants. Various facts and observations have been put forward as evidences to the following categories, such as

Evidences from classification

Morphology (comparative anatomy)

Comparative physiology and biochemistry

Cell biology, embryology, paleontology, and geographical distribution

Evidences From Classification (Taxonomy)

Early classification was largely artificial, because the early systematists considered only the superficial characters for differentiation and classification of the animals and plants. Since the development of the theory of descent with modifications by Lamarck and Darwin attempts have been made to classify the animals on the basis of their interrelationships. A natural classification is an expression of evolutionary relationships between different groups.

It is assumed that organisms in the same group are closely related, while those in separate groups are more distantly related. A natural classification is to reflect evolutionary or phylogenetic relationships. It must be based on homologous structures whose fundamental similarities demonstrate a common ancestry, rather than on analogous structures which happen to bear a superficial resemblance to each other as a result of convergent evolution. Very closely related animals are kept in the same species, which shows that they descend from a common ancestor (Refer Figure 13.1).

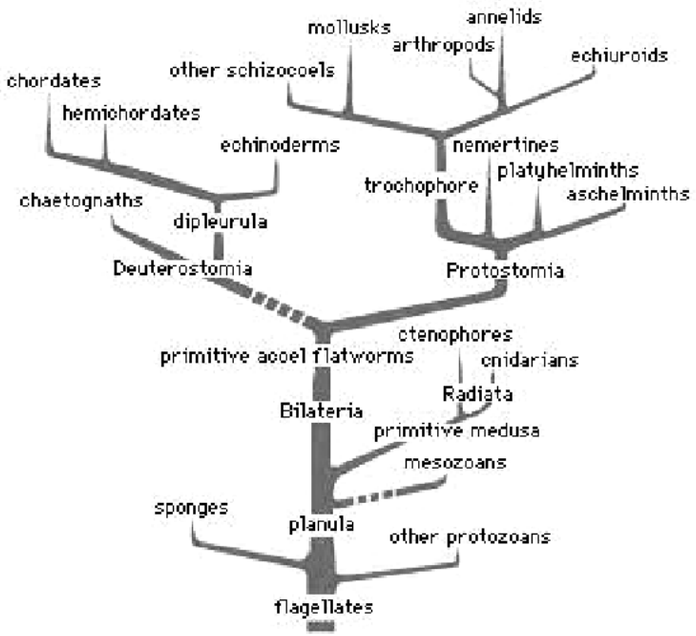

Fig. 13.1 *Schematic Line of Animal Evolution*

The species is the smallest unit of classification, which represents a group of individuals which closely resemble with each other in their morphological, physiological and embryological characters. They are capable of interbreeding among themselves. These become more or less separated from all other co-existing species by the disappearance of intermediate forms. The species which are much alike, are included in one genus, which is distinct from other species, clusters or genera. In the same manner all the like genera are included in one family, which shows again a sign of affinity. Likewise families fall naturally into larger clusters, which are based on similarity, rather than fundamental characters. These like families are called orders. Finally these orders are grouped into classes and the diverse members of the class share only very fundamental characteristics. All the related classes are included in a phylum (Ernst Haeckel). Hence orders, classes and phyla are similarly regarded as they sprung successively from more remote ancestors. The fact that this system of classification, i.e., a given organism resembles some organisms more than the other is possible, and such resemblances show that evolution has occurred. The degree of resemblance is a measure of the remoteness of a common ancestor. In other words, the similarity is an evidence of descent from a common ancestor. The scheme of classification is itself a proof of descent from a common ancestor as shown by the phylogenetic tree. The forms which are associated together in species, genera, classes or phyla are supposed to have descended from a single common ancestor.

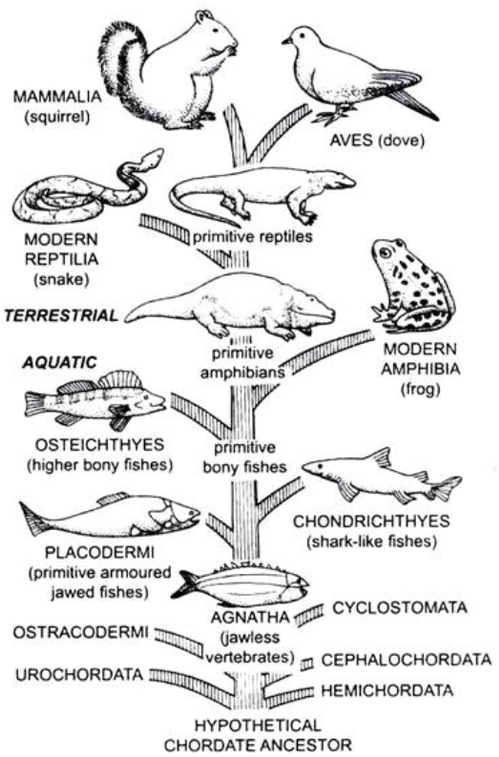

Fig.13.2 *Evolutionary Tree of Chordates based on the Classification*

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Evidences from Morphology and Comparative Anatomy

All animals and plants are alike being composed of protoplasm organized in the form of cells. The various organ systems of vertebrates are constructed on a basic plan and the minor differences visible in some forms are the adaptive modifications to the diverse mode of living reflect common ancestry.

Connecting Links: Animals standing between two groups of animals and separating them from each other are called connecting links, for example, *Euglena* (connecting link between plants and animals) *Proterospongia* (connecting link between protozoa and porifera), *Peripatus* (connecting link between annelida and arthropoda), *Neopalina* (connecting link between annelida and mollusca), *Balanoglossus* (connecting link between non-chordates and chordates), *Chimaera* (connecting link between cartilaginous and bony fishes), *Coelocanth* (connecting link between bony fishes and amphibians), *Archaeopteryx* and *Archaeornis* (connecting link reptiles and birds), and duck-billed platypus (connecting link reptiles and mammals). Here we consider the principle of gradation in structure in existing higher animals (Refer Figure 13.3).

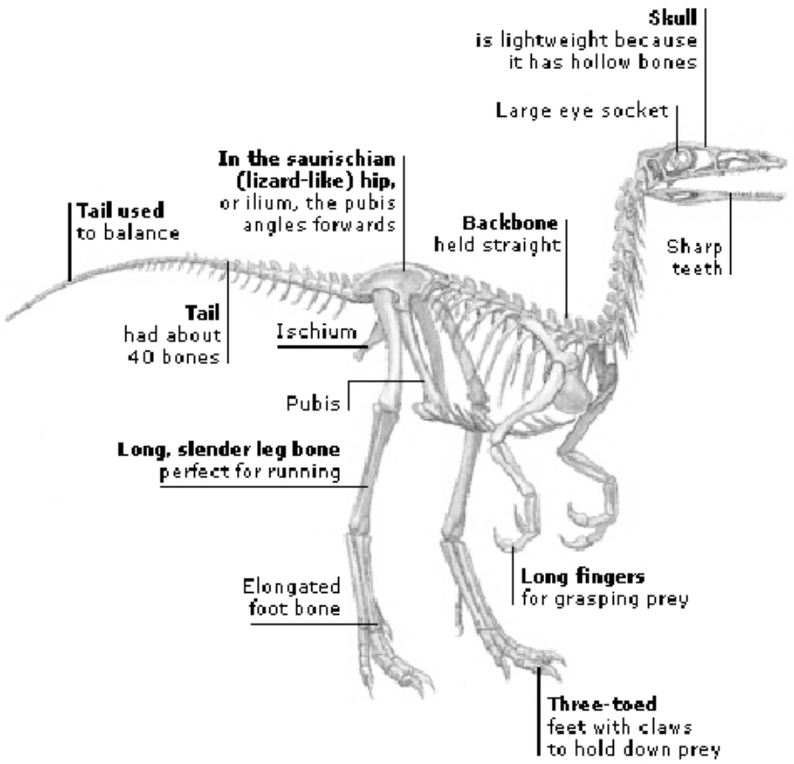

Fig. 13.3 Archaeopteryx *model of Endoskeleton*

For example, consider the widely contrasted groups, the Reptilia and Mammalia. The reptiles are distinguished from mammals by the presence of scaly epidermic covering, lay heavily yolked eggs and do not suckle their youngs, a cloaca into which urinary genital ducts and the alimentary canal open, and have a very complex shoulder girdle. While the mammals have their own contrasted characters, such as epidermic covering of hairs, eggs are very minute and they suckle their youngs, have no cloaca and have separate openings for alimentary canal and urinogenital ducts. The shoulder girdle is not of complex type. There are other mammals belonging to Monotremata (duck-billed platypus and the spiny anteater), which possess hair and suckle their youngs.

Hence, they are classified amongst the Mammalia, but they possess certain characters, due to which they differ from mammals and resemble with the reptiles, such as-they lay large heavily yolked eggs; have a cloaca into which urinogenital ducts and alimentary canal discharge; the shoulder girdle is more like reptiles (presence of interclavicle which is not found in other mammals). Hence these animals occupy an intermediate position between groups. They indicate the paths along which the more highly organized groups have progressed during their evolution from more lowly organized ancestral groups (Refer Figure 13.4).

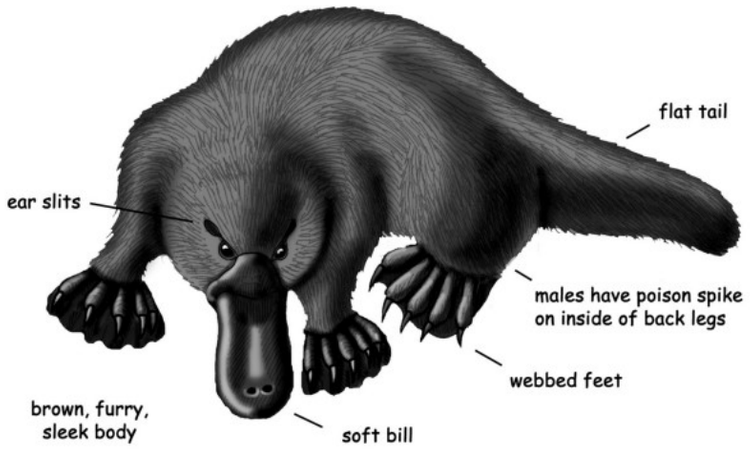

Fig. 13.4 *Duck-Billed Platypus (*Ornithorhynchus*)*

Homology: Homology is the similarity between various organs of different animals and it is based on common embryonic origin or common ancestry. Homologous organs are those which have the common origin and are built on the same fundamental pattern or fundamentally similar. But they perform different functions and have different appearance. These organs are variously modified in adaptation to different functions. The theory of evolution tells that the hereditary characters become gradually modified and these modifications make the organism better suited for the changed conditions of life. For example, pentadactyle forelimb of mammals (Refer Figure 13.5).

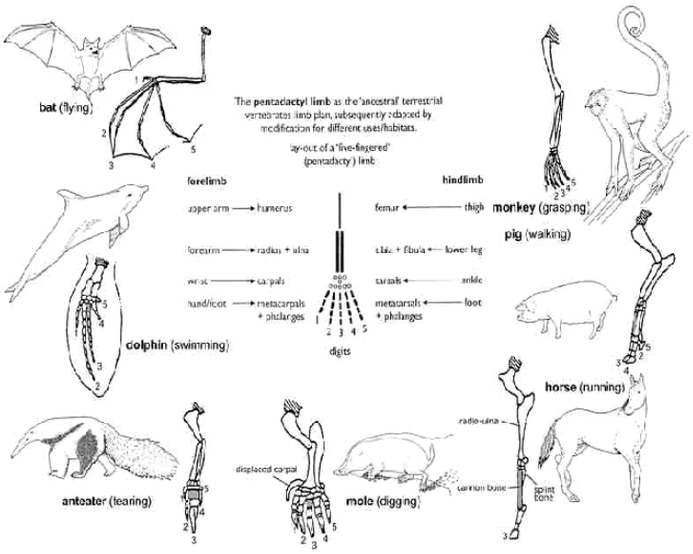

Fig. 13.5 *Principle of Homology Illustrated by Forelimb of Mammals*

The other most common example of homology is the brain. The soft parts of the body present common pattern as do the parts. All the body systems show homology. The brains of vertebrates ranging from fishes to mammals, are constructed of similar parts: olfactory lobes, cerebral hemispheres, optic lobes, cerebellum, medulla and other less prominent divisions and sub-divisions. As we move higher series some lobes becomes more prominent than others. It is due to adaptations and evolutionary change (Refer Figure 13.6).

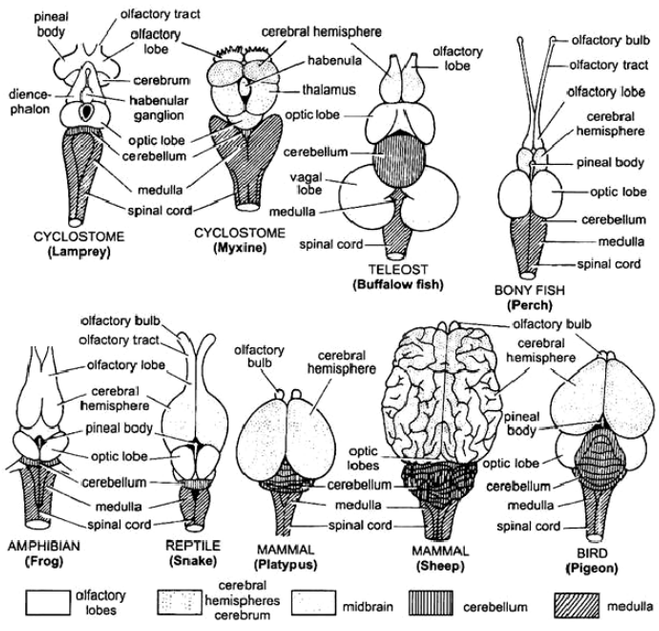

Fig. 13.6 *Principle of Homology Illustrated by Anatomy of Vertebrate Brain*

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There are three categories of homology:

- Phylogenetic homology is that existing between different species (for example, pentadactyl limbs of air-breathing vertebrates),
- Sexual homology exists between sexes of the same species (for example, the testes of a man and ovaries of a woman),
- Serial homology is that existing between organs of the same individual occupying different levels of the body (for example, arm and leg of man and appendages of Crustacea).

Analogy or Homoplasy: Analogous (Homoplastic) organs are those which perform the same function, have superficial resemblance to one another, but are of unlike origin. For example, the wing of insect, the wing of a bird, wing of pterodactyl and wing of a bat doing the same flying function, possess superficial resemblances to each other, but their basic structure different. The wings of bat resemble the arms of a man more than the wings of a bird. Hence it shows that the bats are more closely related to man than birds (Refer Figure 13.7).

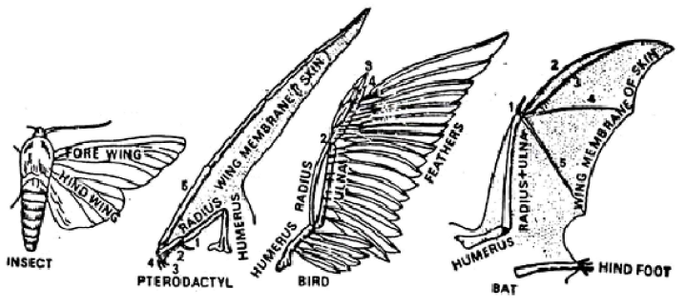

Fig. 13.7 *Principle of Analogy Illustrated between Wings of Insects and Vertebrates*

Similarly the fins of fishes and the flippers of aquatic mammals (whales and seals) serve the same function and have a close superficial resemblance. But structurally they are different. *Ophiosaurus* (lizard), *Uraeotyphlus* (caecilian Amphibia) and *Typhlops* (blind snake), all are analogous cases. They are burrowing in habit, have lost the use of their eyes and loss of limbs. It is an example of convergent evolution and they are not nearly related to one another. The above examples give the evidence for evolution.

Adaptive Radiation: The adaptive radiation shown by the structure of limbs in mammals is also an example as evidence in support of the theory of organic evolution. From the pentadactyl limbs that represent the ancestral plan, numerous lines of evolution led to modification of the basic pattern to serve different functions, thus enabling the descendants to fill a wide variety of ecological niches. The limbs in mammals are adapted for running, climbing, flying, burrowing and swimming etc. This is described as divergent evolution, and clearly it results in adaptive radiation. The end products have certain structural features in common with each other and with the ancestral stock from which they arose. These structural similarities are the basis of homology. Thus the divergent evolution or adaptive radiation is

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representing evolution of new forms in various directions from the common ancestral type (Refer Figure 13.8).

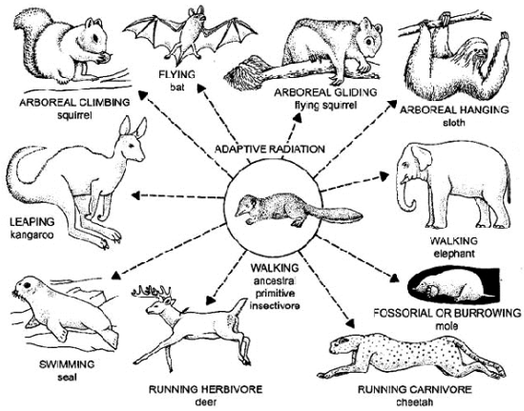

Fig. 13.8 *Diagrammatic Representation of Adaptive Radiation by Divergent Evolution in Mammals*

Adaptive convergence or convergent evolution or parallel evolution produces externally similar structures in organisms (animals) having quite different ancestral origins. Such structures are described as analogous. Generally they perform the same function, though possibly in a very different ways. Any anatomical similarity between them is caused not by a common ancestry, but that structures performing the same function are bound to resemble one another. Examples of analogous structures are the legs of insects and mammals, and the wings of butterflies and birds (Refer Figure 13.9).

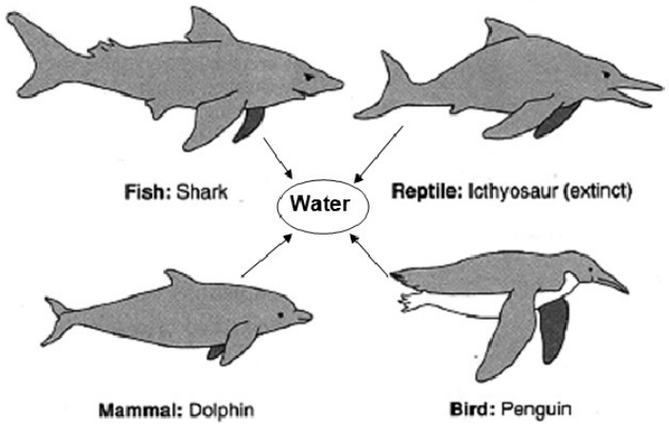

Fig. 13.9 *Principle of Adaptive Convergence or Convergent Evolution Represented by Fish, Reptile, Bird and Mammal*

The legs of insects and mammals, though performing the same tasks, have a quite different structural organization. Similarly, the wings of butterflies and birds, though both used for flight, but are constructed on different principles. Likewise fins and flippers of fishes, reptiles (ichthyosaur), birds (penguin) and mammals

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(whale) attained same forms but different in origin. Thus, they are the products of two distinct lines of evolution, resembling each other only as a result of convergent evolution. Another example of convergent evolution is wings of insects, birds and bat.

Evidences from Vestigial Organs

In some cases evolution has resulted in extreme reduction, even total loss a structure. These are generally useless organs found in many animals ancestors of which may have the same organ in a fully-developed and functional condition. For example, in birds wing, third digit is very much reduced and the fourth and fifth are missing altogether. Structures which are thus, reduced are known as vestigial, and their existence has been used as strong evidence for evolution. It is thought that they performed a normal function in the ancestor, but have since been reduced to such an extent that they have lost their original function. Other examples are: reduced pelvic girdle and tiny rudiments of the hind limbs in *Python*, wings of kiwi of New Zealand, and reduced muscles of the external ears, coccyx, vermiform appendix, vestigial mammary gland, nictitating membrane in eyes and wisdom teeth (3rd molars) of man (Refer Figure 13.10).

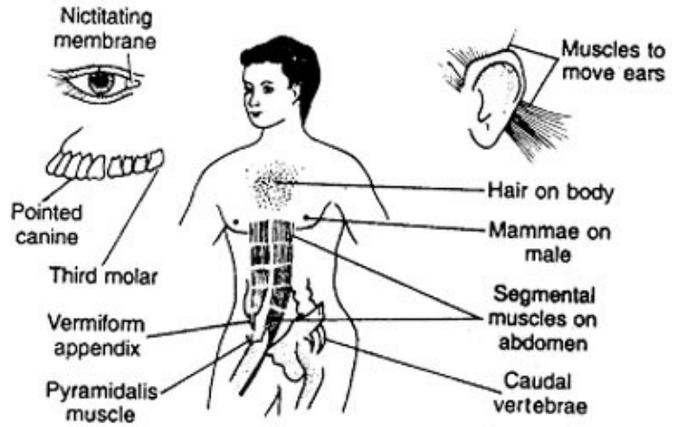

Fig. 13.10 *Vestigial Organs in Man showing Evidences of Evolution*

Evidences from Comparative Physiology and Biochemistry

For the establishment of evolutionary evidences, physiological processes and the chemical composition of various cells and tissues of different animals have been compared. The similarities between different animals, as regards their physiology and chemical composition, are considered as evidence of relationship which show that they have descended from a common ancestor. For example, the protoplasm in all organisms is basically the same. It mainly consists of proteins, carbohydrates, fats, water, etc. It suggests that during evolution the most fundamental property of living being has remained intact. Similarly the nucleic acid (DNA) found in chromosomes with other basic proteins (histones and protamines) are also similar

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in all the organisms. Other proteins like globulins and some amount of RNA are also found associated with chromosomes. Such type of uniformity in the compositions chromosomes suggests a common origin of all the living beings. The comparisons of sequence of bases in the DNA of different organisms also determine the possible evolutionary relationships. The more alike sequences, the closer the organisms are presumed to be in evolution. The other most common examples are enzymes and hormones. The very similar enzymes and hormones are found in large groups of animals. For example, trypsin is found from Protozoa to mammals; amylase is found from sponges to man and thyroxin is found in all vertebrates and it has been proved to be interchangeable among them. Beef thyroid is used successfully in the treatment of human thyroid deficiencies. This hormone is also essential for the metamorphosis of frogs. The blood relationship is also considered as evidence of evolution (serological evidence). The phylogenetically closer two species have their blood proteins more alike. Demonstrating the closeness of animals involves the use of serological tests. It brought man close to monkeys and apes (Refer Figure 13.11).

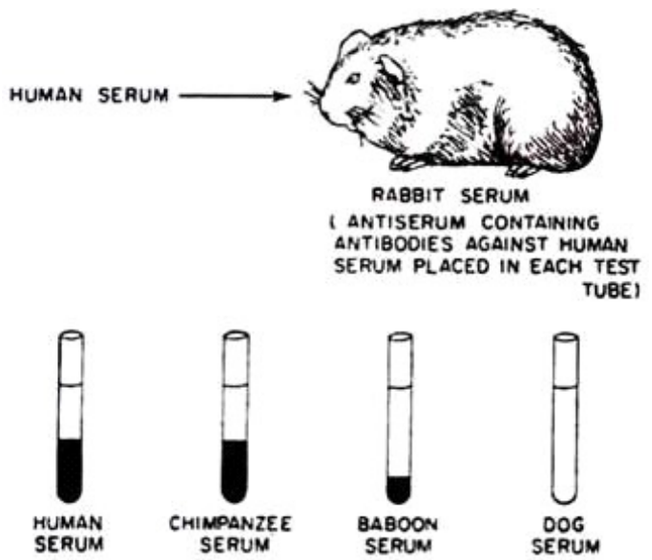

Fig. 13.11 *Precipitin based Serological Test showing Evolutionary Relationship among Organisms*

Evidences from Embryology

Embryology is the study of development of animals from egg to adult. The embryology also provides evidence for the theory of evolution. For instance, all the multicellular animals begin their life as a single fertilized cell (comparable to a Protozoa) and by repeated divisions, it produced the blastula (common stages for all many-celled animals), which later on produced two layered gastrula (nearly common for all many-celled animals). The outer layer of gastrula is the future ectoderm and the inner one is the future endoderm lining the archenteron, the future digestive tract. After gastrula stage, the development became modified in

different groups of animals. In a few many-celled animals, the embryo hatches either just after the gastrula stage or before that. But in the higher animals, the development takes place within the egg or within the body of the mother up to the formation of a complex-bodied embryo or young animal. When we compare the developmental stages of starfish, a beetle, a fowl and a human, all are found to be alike in the beginning through several stage up to gastrula. Later, the embryos begin to diverge from each other (Refer Figure 13.12).

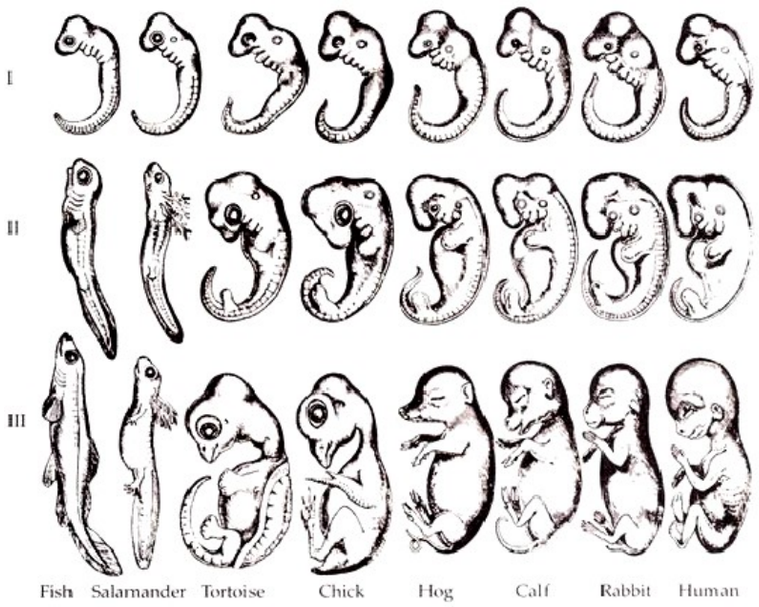

Fig. 13.12 *Three Stages of Development in Vertebrates showing Evidences of Evolution*

Annelids include all the segmented worms and molluscs on the other hand, contain the totally unsegmented snails, but in their early development they are remarkably similar in pattern of cleavage, arrangement of blastomeres and formation of membrane and coelomic cavities, and both the animals share a so called trochophore larva, a little creature with a a characteristic girdle of cilia and a number of other diagnostic features. The aforesaid characteristics provide an evidence of common ancestry between Annelida and Mollusca. Naturalists believe that all vertebrate animals are related to each other and have descended from a common ancestor. The tadpole stage shows resemblance with a fish during development and later on becomes adult Amphibia. On the basis of the developmental history of the animals, Ernst Haeckel suggested that during its embryological development an organism repeats its ancestral history. Thus, he postulated his famous recapitulation theory or biogenetic law, i.e., 'ontogeny (embryonic development of an organism) recapitulates phylogeny (evolutionary history of the race of phylum)'. This simply means that embryos, in their development, repeat the evolutionary history of their ancestors in an abbreviated form, for example, the ontogeny of man indicates a long and complicated history.

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Evidences from Paleontology

Paleontology gives the direct evidence that evolution has taken place Paleontology deals with the study of fossil remains of extinct animals or their traces of existences. Fossils are only animals and plants which have been dead rather longer than those, which died yesterday (T.H. Huxley). Fossils are generally preserved in sedimentary rock, which is formed by the deposition of silt, sand or calcium carbonate over millions of years. Silt deposits give shales, sand gives sand stone, and calcium carbonate (which may be precipitated from solution or derived from the shells of animals) gives limestone. Sedimentary rock is comparatively soft. Fossils found in sedimentary rocks formed by the deposition of sand or mud on the bottom of lakes or sea. These rocks are formed one above the other forming columns of rocks arranged in sequence. Naturally the bottom layers represent the oldest rocks and the most upper ones are the newly formed rocks. Thus, the fossils present in the bottom layers of the rocks will have existed in the past than those which are found in the rocks lying above them. Thus, fossils arranged according to their age, tell the story of evolution. There is a gradual progression towards higher types, when proceed from the lower to the higher strata or geological periods.

The evolutionists believe that many fossils are quite different from the forms found today showing that evolution has taken place. For example, Jurassic *Archaeopteryx* and *Archaeornis* were first fossil birds which are quite different from the modern birds. They show few reptilian characters, such as long tail, jawed teeth, solid bones, and few avian characters, such intermediate forms have not been preserved, which separate the two groups of animals. Such forms are called missing link. The most common and important example of fossil series is the evolution and modification of modern horse. The first horse like animal appearing in Lower Eocene of Europe was the *Hyracotherium*. Another Lower Eocene form was the *Eohippus*, which lived in North America and it was probably migrated from Asia to North America by the Alaskan land connection. Then came in this series the *Orohippus* (Upper Eocene), *Mesohippus* (Lower Miocene). *Pliohippus* (Upper Pliocene) and finally the *Equus* of Pliocene and Pleistocene. These intermediate forms from *Eohippus* to modern *Equus* have now discovered from geological strata. The evolution of horse clearly demonstrates the evolution theory. Due to certain climatic condition structural changes took place in the horses of ancient or ancestral period for adapting themselves to changed conditions. For example, limbs became greatly elongated, reduction from four-toed condition to one, cheek teeth became adapted for grazing, neck and head became elongated. All these changes have taken place gradually (Refer Figure 13.13).

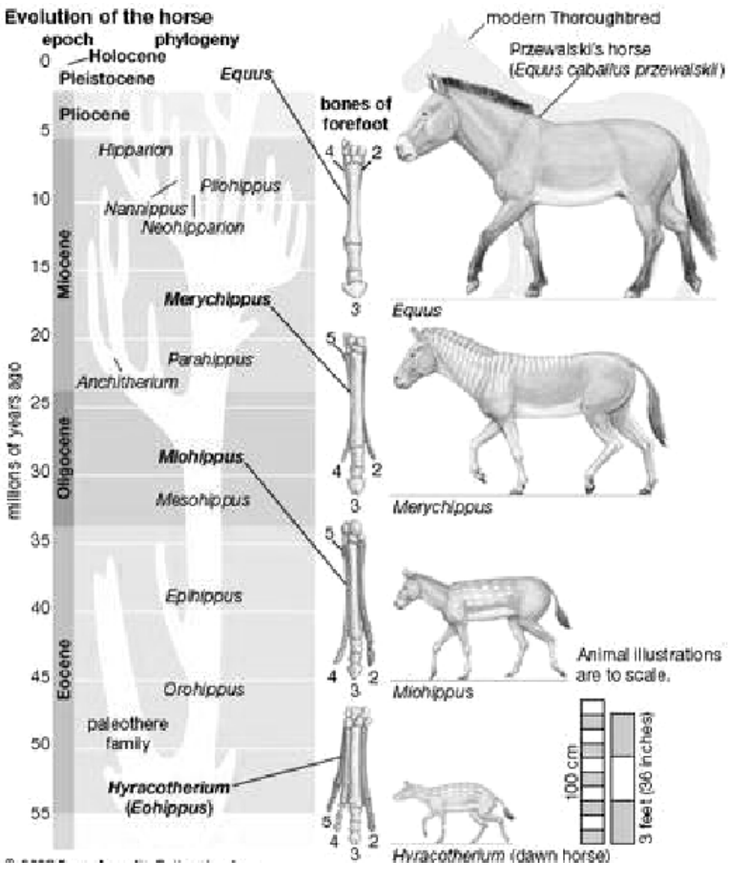

Fig. 13.13 *Paleontlogical Evidences for Evolution and Phylogeny of Horse*

Check Your Progress

1. What is a species?
2. Define races.
3. Define allopatric populations.
4. Write about sympatric populations.

13.3 SPECIATION

In taxonomy, a species represents the lowest organism (living being). Species is a genetic unit having large intercommunicating gene pool and it occupies a specific ecological niche, that is unoccupied by another closely related species. Species have fully effective reproductive isolating mechanisms. Mechanisms that prevent gene exchange have been broadly term isolating mechanisms. Some authors include in this category all factors that prevent gene exchange-geographical and spatial isolation. Such geographically isolated populations, also called allopatric

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populations do not have the opportunity for gene exchange. Whether many of them would still remain reproductively isolated if they have given opportunity to mix. Other authors have, therefore, proposed that isolation mechanisms to be restricted those that prevent gene exchange between populations in the same geographic locality, i.e., mechanisms that isolate sympatric populations. Thus the process of splitting a genetically homogeneous population into two or more populations which undergo genetic differentiation and reproductive isolation is called speciation. Species originate in many ways as given below:

Phyletic or Autogenous or True Speciation

In phyletic speciation one species is transformed into other species in a long period of time by sBow and steady changes. This is transformation of species in due course of time. This transformation is due to:

- Adaptations to a change environment
- Increasing speciation for a particular environment
- Improved adaptations in a constant environment

In phyletic speciation, evolving species presents a line of succession in which one is replaced by the other (Refer Figure 13.14).

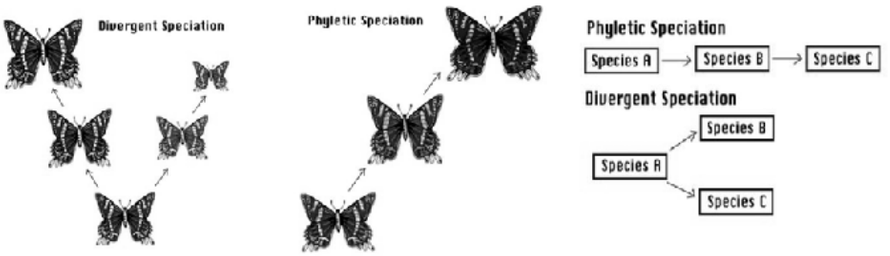

Fig. 13.14 *Schematic Representation of Phyletic and Divergent Speciation*

Quantum Speciation

It represents accelerated pace of phyletic gradualism. It involves accumulation of genetic changes in relatively rapid succession. Here chromosomal rearrangements produce reproductive isolation resulting into speciation. These evolutionary events occur suddenly and intermittently. Quantum speciation may occur due to chromosomal aberrations (inversion and translocation) or change in chromosome number (polyploidy, autopolyploidy and amphidiploidy). Michael White studied evolution in flightless grasshoppers, in which one of its small population, a translocation occurred stochastically. Such translocation heterozygotes were found to have some reduced fitness perhaps due to abnormal meiosis. Its translocation homokaryote with two copies of translocation chromosomes or two normal chromosomes, were favoured by selection because of producing normal offsprings. These translocation homokaryotes having a chromosome number less than normal established them as distinct semispecies, because their hybrids with ancestral

population had unbalanced chromosome complement and had established reproductive isolation. This is also called as statispatric speciation. Here both autoploidy and allopolyploidy have played major role in the origin of new species from the pre-existing species (Refer Figure 13.15).

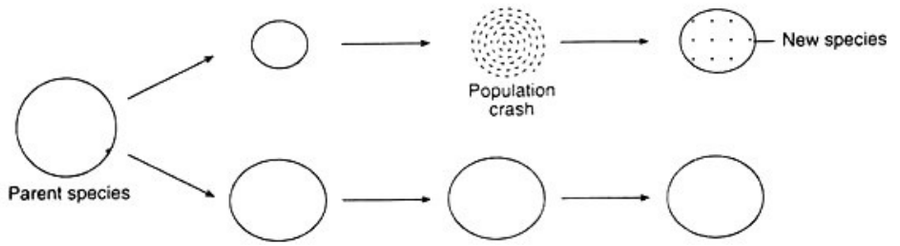

Fig. 13.15 *Mechanism of Quantum Speciation*

Gradual Speciation

Speciation is a microevolution process. This occurred due to gradual accumulation of many minute gene differences over a long period of time under the influence of natural selection. In gradual speciation, one species gives rise to one or more new species. This is of three types:

Allopatric Speciation (Geographical Speciation): New species arise when some physical geographical barrier divides the large population of a species into two or more small populations. Individuals of these isolated populations cannot interbreed. Geographic barrier obstructs free gene flow between the different populations. Each new population accumulates differences and thus, evolves independently into species (Refer Figure 13.16). The allopatric speciation can be divided into three stages:

- Formation of isolated populations by geographical barriers
- Genetic divergence due to persistence of isolated populations and their differentiation from the parental population
- Speciation, i.e., establishment of reproductive isolation between new populations that forms new species

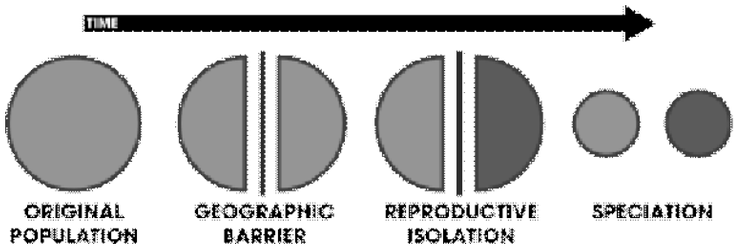

Fig. 13.16 *Process of Allopatric Speciation*

Sympatric Speciation: The evolution of new species within one locality is called as sympatric speciation. The establishment of new populations of a species in different ecological niches within normal range of parental population is called sympatric speciation. Sympatric speciation can occur by a rapid development of reproductive isolation between members of the population in different niches. The rapid reproductive isolation can arise either due to changes in the chromosome number or due to introgressive hybridization or polyploidy. The change in the chromosome number may occur by lyploidy, aneuploidy, haploidy or translocation. Origin of species by these methods are quite frequent in plants and are few in animals. Crop plants like wheat, oats, cotton, tobacco and sugarcane have evolved by sympatric speciation. Different species of *Drosophila* have different number and appearance of chromosomes: *D. melanogaster* and *D. americana* have 4 pairs of chromosomes; *D. virilis* has 6 pairs of chromosomes; *D. pseudo-obscura* and *D. persimilis* have 5 pairs of chromosomes and *D. willistoni* have 3 pairs of chromosomes. The chromosomal composition of *D. virilis* is regarded as ancestral type (Refer Figure 13.17).

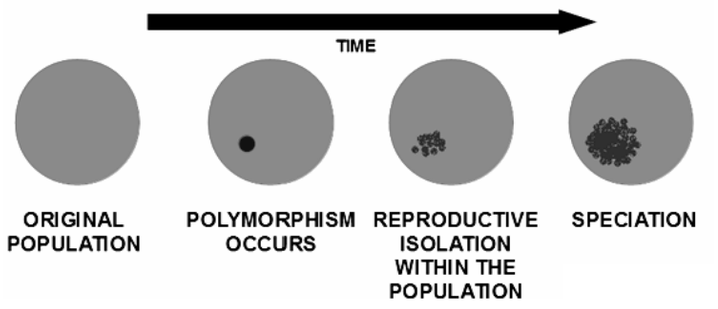

Fig. 13.17 *Mechanism of Sympatric Isolation*

Parapatric Speciation: Development of reproductive isolation among members of a continuous population in the absence of geographical barrier is called parapatric speciation. Parapatric populations coexist only in one or more overlapping regions at the peripheries of their geographical distribution. White proposed that chromosomal aberrations lead partial reproductive isolation in the individuals of a population in some areas of its distribution. The lower fertility and structural changes in the chromosomes finally establishes reproductive isolation forming new species. For example, land snails, *Partula* with 11 species on the island of Moorea near Tahiti.

Peripatric Speciation: Peripatric speciation is a special version of the allopatric speciation mode and happens when one of the isolated populations has very few individuals. Peripatric speciation is a mode of speciation in which a new new is formed from an isolated peripheral population. Since peripatric speciation resembles allopatric speciation, in that populations are isolated and prevented from exchanging genes, it can often be difficult to distinguish between them. Nevertheless, the primary characteristic of peripatric speciation proposes that one of the populations is much

smaller than the other. The terms peripatric and peripatry are often used in biogeography, referring to organisms whose ranges are closely adjacent but do not overlap, being separated where these organisms do not occur, for example on an oceanic island compared to the mainland. Such organisms are usually closely related (for example, sister species); their distribution being the result of peripatric speciation. The concept of peripatric speciation was first outlined by the evolutionary biologist E. Mayr based, other alternative models developed such as centrifugal speciation (Refer Figure 13.18).

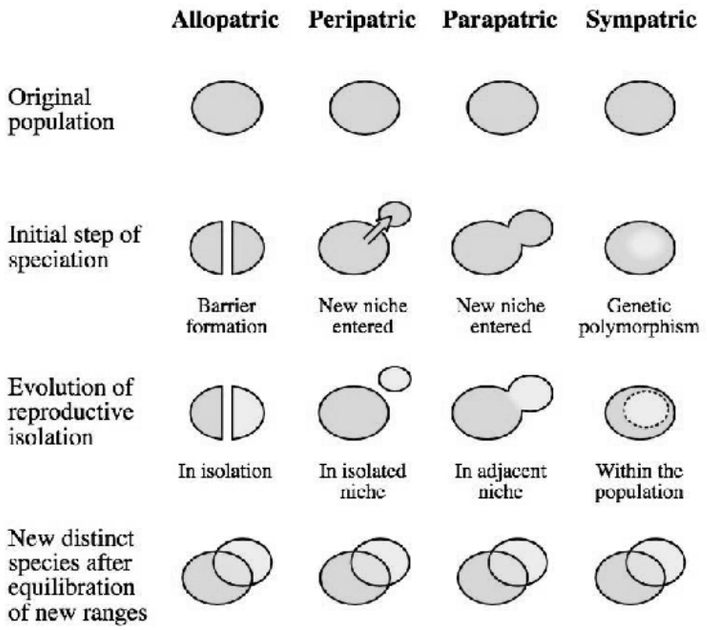

Fig. 13.18 *Comparative Accounts of Different Gradual Speciation*

Patterns of Behavioral Adaptation

Wallace proposed that natural selection might favor for the establishment of mating barriers between populations if the hybrids were inferiorly adapted. It means that genotypes which did not mate to produce inferior hybrids would be selected in comparison to those genotypes that mate to produce inferior hybrids. Thus, selection for sexual isolation arises because most races and species are strongly adapted to specific environments. Thus, different populations producing deleterious hybrids be exposed to each other in the same locality. Thus, more sexually isolated genotypes be specifically isolated. Therefore, speciation should occur in the following sequence:

- Genetic differentiation between allopatric populations
- Overlap of differentiated populations in a sympatric area
- Selection for increasing sexual isolating mechanisms

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Sexual isolating mechanism should be strongest among sympatric populations, since they are sufficiently close to produce deleterious hybrids, and weakest among allopatric populations that are too distant to produce such hybrids. Grant has reported that of nine species in *Gilia*, those that are most difficult to cross are the sympatric ones. Allopatric species show no barriers against hybridization although all the crosses are sterile.

Allopatric populations even accumulate a sufficient number of genetic differences and show sexual isolation when they are brought together in the same locality. The species barriers breakdown produce viable and fertile hybrids zones of hybridization or hybrid swarms may occur. Their genotypes and phenotypes are intermediate to both parental species. If a unique and discrete habitat exists into which hybrids are better adapted than the parents, new population may eventually become isolated from the parental population. A population in a single locality selected for adaptation to different habitats within that locality could produce an increase in genetic viability that would lead to polymorphism (Mather and Thoday). Such polymorphism is found in British peppered moth. Populations distributed over a wide geographical range or have occupied well separated geographic habitats for a long time, usually show distinct morphological phenotypic) differences. These differences are acquired in adaptation to climatic factors. These phenotypic differences are controlled genetically. Such populations of a species showing phenotypic and genotypic differences due to climate are called geographic races.

Mechanism of Isolation in Speciation

May has classified sympatric isolating mechanisms into two broad categories:

- Premating isolating mechanisms
- Postmating isolating mechanisms

Premating Isolating Mechanisms

There are following mechanisms operating among premating isolating mechanisms:

- **Seasonal or Habitat Isolation:** In this case, potential mates do not meet because they flourish in different seasons or in different habitats. *Tradescantia canaliculata* and *T. subaspera* (plants) are sympatric throughout their geographical distribution. They are isolated because their flowers bloom at different seasons. One species grows in sunlight and the other in deep shade.
- **Behavioral or Sexual Isolation:** Here sexes of two species of animals may be found in the same locality at the same time, but their courtship patterns are sufficiently different to prevent mating. The distinctive songs of many birds, special mating calls of certain frogs and sexual displays of most animals (e.g., peacock), are generally attractive only to mate of the same

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species. Many plants have floral displays for attracting insect pollinators. Where morphological differences between two species is minimum, behavior differences may be enough to prevent cossfertilization. Thus, *Drosophila melanogaster* and *D. simulans,* called sibling species due to morphological similarity, do not mate normally with each other although kept together in a same cage.

- **Mechanical Isolation:** Here mating is attempted, but fertilization cannot be achieved due to differences in genital organs. In Damsel fly species and some other groups are the exceptions in which mating occurs.

Postmating Isolating Mechanisms

These prevent interpopulational cross even though mating occurs due to:

- **Gametic Mortality:** In this case either sperm or egg is destroyed because of interspecific cross. In plants, pollen grains do not grow in pollen tubes in the styles of foreign species. In some Drosophila crosses, insemination reaction takes place in female vagina that causes swelling of vagina and prevents fertilization of egg.
- **Zygotic Mortality and Hybrid Inviability:** Here egg is fertilized but zygote either does not develop or develops into an organism with reduced viability. Moore made crosses between 12 frog species of genus *Rana* and found wide range of inviability. In some crosses, cleavage of egg was not observed. In others, cleavage and blastula stages were normal but gastrulation failed. In others, early development was normal but later stages could not develop.
- **Hybrid Sterility:** Here hybrid has normal viability but is reproductively deficient or sterile. It is found in mule and many other birds. Hybrid between male donkey and female horse is called mule. It is superior to both parents in fitness, yet it is almost sterile. Sterility in such cases may be caused by interaction between genes from different sources. Dobzhansky has shown that the genetic factors causing sterility are located on all chromosomes. A chromosomal interaction takes place in hybrid males. The greater the difference in species origin between sex chromosomes and autosomes, the greater the degree of sterility.

Check Your Progress

5. Give name of animal showing characters of reptiles and aves.
6. What are vestigial organs?
7. Give an example of homologous structures in vertebrates.

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13.4 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. A species is a particular morphological type distinctly different from the morphological types of other species.
2. Populations of the same species that differ markedly from each other are called races.
3. The Geogrphically isolated populations amongst which gene exchange do not occur are allopatric populations.
4. The species living in the same geographic locality but there is no gene exchange between them are known as sympatric populations.
5. *Archaeopteryx* animal show characters of reptiles and aves.
6. Useless organs having no function in the body, for example, wings of flightless birds, vermiform organs in man are called as vestigial organs.
7. Fore limbs of mammals, brain of vertebrates are the example of homologous structures in vertebrates.

13.5 SUMMARY

- The remains or traces of organisms from a past geologic age embedded in rocks by natural processes are called fossils. They are extremely important for understanding the evolutionary history of life on Earth.
- Biologists believed that a species was ideally represented as a particular morphological type distinctly different from the morphological types of other species. According to Darwin, morphological species types arose by Natural selection among the variable members of previous species.
- There are convincing proofs about the existence of evolutionary mechanisms in living animals and plants. Various facts are: Evidences from classification, morphology (comparative anatomy), comparative physiology and biochemistry, cell biology, embryology, paleontology, and geographical distribution.
- Homologous organs are those which have the common origin and are built on the same fundamental pattern or fundamentally similar but morphologically may different. But they perform different functions and have different appearance. For example, pentadactyle forelimb of mammals.
- Analogous (Homoplastic) organs are those which perform the same function, have superficial resemblance to one another, but are of unlike origin. For example, the wing of insect, the wing of a bird, wing of pterodactyl and wing of a bat doing the same flying function, possess superficial resemblances to each other, but their basic structure different.

- The adaptive radiation shown by the structure of limbs in mammals is also an example as evidence in support of the theory of organic evolution. The limbs in mammals are adapted for running, climbing, flying, burrowing and swimming etc. This is described as divergent evolution, and clearly it results in adaptive radiation.
- Adaptive convergence or convergent evolution or parallel evolution produces externally similar structures in organisms (animals) having quite different ancestral origins. Examples of analogous structures are the legs of insects and mammals, and the wings of butterflies and birds.
- Paleontology deals with the study of fossil remains of extinct animals or their traces of existences. Fossils are only animals and plants which have been dead rather longer than those, which died yesterday (T.H. Huxley). For example, in modern horse limbs became greatly elongated, reduction from four-toed condition to one, cheek teeth became adapted for grazing, neck and head became elongated.
- New species arise when some physical geographical barrier divides the large population of a species into two or more small populations, called allopatric speciation.
- The evolution of new species within one locality is called as sympatric speciation.
- Development of reproductive isolation among members of a continuous population in the absence of geographical barrier is called parapatric speciation.
- Peripatric speciation is a special version of the allopatric speciation mode and happens when one of the isolated populations has very few individuals. Peripatric speciation is a mode of speciation in which a new is formed from an isolated peripheral population.

13.6 KEY WORDS

- **Species:** A species is a particular morphological type distinctly different from the morphological types of other species.
- **Races:** Populations of the same species that differ markedly from each other are called races.
- **Allopatric populations:** The Geogrphically isolated populations amongst which gene exchange do not occur are allopatric populations.
- **Sympatric populations:** The species living in the same geographic locality but there is no gene exchange between them are known as sympatric populations.
- **Vestigial organs:** Useless organs having no function in the body, for example, wings of flightless birds, vermiform organs in man are called as vestigial organs.

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13.7 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short Answer Questions

1. Give the evidences of evolution from morphology and comparative anatomy.
2. Brief a note on evidences of evolution from Embryology.
3. Give short note on phyletic or autogenous or true speciation.
4. What is quantum speciation?
5. Write a brief note on gradual speciation.
6. Give a short descriptive note on patterns of behavioral adaptation.

Long Answer Questions

1. Describe in detail about species categorization.
2. Write an essay on species and speciation.
3. Discuss isolation and mechanism of isolation for speciation.
4. Describe the evidences of organic evolution from comparative morphology and anatomy.
5. Write an essay on evidences of evolution based on embryology, biochemistry and physiology.
6. Discuss in detail the evidences of organic evolution through taxonomy and paleontology.

13.8 FURTHER READINGS

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UNIT 14 GENE: FUNDAMENTAL CHARACTERISTICS AND MOLECULAR VARIATIONS

Structure

14.0 INTRODUCTION

Man is clearly a mammal. Hence the evolutionary history of mammals in general is the evolutionary history of man. Briefly we recall that sequence as follows: crossopterygian fishes, to labyrinthodont amphibians, to cotylosaur reptiles, to therapsid reptiles, to primitive mammals of group Pantotheria, to mammals of order Insectivora (shrews, moles, hedgehogs and alamiqui). From the insectivores the other orders of placental mammals are believed to have arisen. Among these other orders is order Primates, to which man belongs, in company with tree shrews, lemurs, tarsiers, monkeys and apes. These forms are grouped together in one order because they possess a number of common anatomical characters. Members of order primates exhibit a trend towards freeing of the forelimbs from locomotor duties, notably for grasping and handling of objects. The lower primates show only the beginning of this trend, which reaches its culmination in man. This trend was of highest importance in human evolution being closely connected with development of the upright posture, which is the most distinctive character of man.

Molecular phylogenetics predates DNA sequencing by several decades. It is derived from the traditional method for classifying organisms according to their similarities and differences, as first practiced in a comprehensive fashion by Linnaeus in the 18th century. Linnaeus was a systematicist not an evolutionist, his objective being to place all known organisms into a logical classification which he believed would reveal the great plan used by the Creator- the *Systema Naturae*.

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In this unit, you will study about the mutation theory of evolution, population genetics and gene pool, origin, evolution and characteristics of modern man and concept and application of phylogenetic analysis in detail.

14.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand the mutation theory of evolution
- Explain the population genetics and gene pool
- Discuss about origin, evolution and characteristics of modern man
- Describe the concept and application of phylogenetic analysis

14.2 MUTATION THEORY OF EVOLUTION

C. Darwin recognized two kinds of variations as material for natural selection. These variations were mainly ever present fluctuating (continuous), which played dominant role in species formation. Besides, there were other discontinuous variations appearing occasionally in the generations, which were termed as 'sports or saltatory variations or mutations'. These sports occurred rarely and Darwin considered them of little importance. Mutations are regarded as the ultimate source of new and different genetic material appearing in a population, or the genetic mechanism producing the change is known as a mutation. Mutations are the basis of discontinuous variation in populations. Mutations arise spontaneously and are not directed by the environment. Environmental influences can affect the mutation rate, but they cannot induce a particular mutation to take place. From the evolutionary stand point, the only direct part played by the environment is in selecting mutants that happen to possess advantageous characteristics.

Hugo de Vries (a well-known Dutch Botanist) in Holland gave much importance to these discontinuous variations (sports or saltatory variations) and described that new species arise not by the accumulation of minute fluctuating variations through natural selection, but by these suddenly appeared saltatory variations (mutations). The term mutation was for large spontaneous inheritable changes, which occur suddenly in naturally reproducing populations. Mutation is a hereditary change in the genetic make-up of an organism other than that, which may be caused by the simple recombination of genes. These include the changes in the gene structure or composition (gene mutations or point mutations) and the changes in the chromosomes either in number or structure (chromosomal mutations).

Man is clearly a mammal. Hence the evolutionary history of mammals in general is the evolutionary history of man. Briefly we recall that sequence as follows: crossopterygian fishes, to labyrinthodont amphibians, to cotylosaur reptiles, to therapsid reptiles, to primitive mammals of group Pantotheria, to mammals of order Insectivora (shrews, moles, hedgehogs and alamiqui). From the insectivores

the other orders of placental mammals are believed to have arisen. Among these other orders is order Primates, to which man belongs, in company with tree shrews, lemurs, tarsiers, monkeys and apes. These forms are grouped together in one order because they possess a number of common anatomical characters. Members of order primates exhibit a trend towards freeing of the forelimbs from locomotor duties, notably for grasping and handling of objects. The lower primates show only the beginning of this trend, which reaches its culmination in man. This trend was of highest importance in human evolution being closely connected with development of the upright posture, which is the most distinctive character of man.

Molecular phylogenetics predates DNA sequencing by several decades. It is derived from the traditional method for classifying organisms according to their similarities and differences, as first practiced in a comprehensive fashion by Linnaeus in the 18 century. Linnaeus was a systematicist not an evolutionist, his objective being to place all known organisms into a logical classification which he believed would reveal the great plan used by the Creator- the Systema Naturae. However, he unwittingly laid the framework for later evolutionary schemes by dividing organisms into a hierarchic series of taxonomic categories, starting with kingdom and progressing down through phylum, class, order, family and genus to species. The naturalists of the 18th and early 19th centuries likened this hierarchy to a 'tree of life', an analogy that was adopted by C. Darwin in The Origin of Species as a means of describing the interconnected evolutionary histories of living organisms. The classificatory scheme devised by Linnaeus therefore became reinterpreted as a phylogeny indicating not just the similarities between species but also their evolutionary relationships.

Dutch Botanist Hugo de Vries was born at Haarlem, studied at Heidelberg and also Wurgberg. He was a university lecturer at Amsterdam and also held the professorship of Plant Physiology. His book entitled '*Die Mutation Theorie*' was published in 1901. In this book, de Vries proposed the mutation theory in order to explain the mechanism of evolution. This theory was based on his observations on evening primrose, *Oenothera lamarckiana* grown in a field near Amsterdam. He studied this plant in wild form for many years continuously and spontaneous changes in some of these wild plants. These plants differed considerably in stem height, flower color and leave's shapes. He observed that these changes were heritable and ultimately several new varieties. He succeeded in cultivating all these new varieties and named them as mutant varieties. In fact, he selected for his breeding experiments two mutant varieties: *Oenothera laevifolia* characterized by smooth leaves and *O. brevistylis* characterized by short styles. And he observed that these features were breeding true and so, he regarded these mutant strains as the distinct species. Thus, de Vries recognized the following seven distinct species of *Oeonothera*:

- *O. gigas* (giant and stout plants with large flowers and deep green leaves)
- *O. rubrinervis* (fruit red veined, leaves pale green and stem slender and britle)

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- *O. oblonga* (dwarf and weak plants with oblong leaves)
- *O. albida* (weak plants with whitish-pale leaves)
- *O. laevifolia* (leaves narrow and smooth and pale flowers)
- *O. brevistylis* (round leaves, flowers with very short style and flattened stigma)
- *O. vanilla* (very short or dwarf variety; leaves sessile)

According to de Vries, these characters were originated suddenly and were inheritable. The plants with new characters transmitted them to their offsprings, and de Vries called them as mutants and the newly developed characteristics were called mutations. On the study of these forms, the theory of mutations was formulated. According to this theory, new species originate as a result of these large, discontinuous variations appeared suddenly and were fully developed (Refer Figure 14.1).

The characteristics of the theory of mutation are as given below:

- Mutations appear from time to time among the organisms of a naturally breeding species or populations. The organisms with mutations are called mutants. These mutants are clearly distinct from their parents.
- Mutations are heritable and form new species. They do not disappear by crossing.
- Mutations are sudden and large and are different from Darwin's fluctuating variations, which are small and directional.
- Mutations may appear in any direction, and are subjected to natural selection.
- Unsuitable mutants are destroyed by natural selection.
- Mutations appear full-fledged and hence there is no question of incipient stages in the development of an organ.
- Mutation occurs in all possible direction and may be advantageous or disadvantageous.

Fig.14.1 *Flower of Evening Primrose*

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Characteristics of Mutations

The de Vries theory of mutation has following characteristics:

- Mutations are changes in the genes or chromosomes.
- Mutations may occur at any stage in the development of an organ. These can be somatic or germinal. Somatic mutations are not heritable. Germinal mutations occur in the cells of sex organs or at the time of maturations of gametes or in the mature gametes.
- If mutation occurs in one mature gamete, it will appear in one individual of the progeny and it will be observable only if it is dominant.
- A dominant gene mutation in one of the two daughter chromosomes at the first division of the zygote will develop half of the parts showing mutated characters and the remaining half showing parental character.
- Mutations may be dominant or recessive. The dominant gene mutations are at once visible, while the recessive gene mutations remain hidden for several generations.
- Mostly mutations are recessive and deleterious. They may be lethal also.
- Mutated gene may affect more than one character (pleiotropic). For example, white eye mutant of Drosophila not only changes the color of eye from red to white, it also causes transparency of the testicular envelope, change in spermatheca shape, lowered viability, fertility, etc.
- Mutations are of all magnitudes. Mutations with marked effects are less frequent than those with slight effects.
- Mutations do not always involve the same characters and hence are not directional.
- Mutational changes affect all body organs, their physical, physiological and even biochemical traits.
- Mutations occur at random. They may be useful to the organisms or not.
- Mutations are universally found in all the organisms from virus to man.
- Mutations only do not produce new species as speculated by de Vries.

Advantages of Mutation Theory

Some of the advantages of mutation theory are briefly mentioned below:

- The mutation theory describes the importance of mutation in selective value of organisms.
- Mutation explains the occurrence of evolutionary changes within short period in contrast to natural selection, which describes slow and continuous variations.
- Mutation theory also explains the absence of connecting links as no criteria against evolution but its possibility exists.
- This theory provides a great service to breeders in developing new useful varieties.

- Occurrence of mutations in large and divergent directions removes the possibility of species disappearance by crossing, etc.
- The differences between related species consist largely in difference of unimportant organs as explained by mutation theory.
- Since mutations appear fully formed from the beginning, there is no difficulty in explaining difficulty in incipient stages in the development of organs.

Criticism of Mutation Theory

Now a day mutations are noticed among bacteria, bacteriophages, viruses and also in men and other organisms. the theories de Vries mutations were significant to the evolutionary process. Though mutations alone are not possible for evolution, but these provide the raw material on which other forces can act to bring about the evolutionary changes. The mutation theory unable to explain properly following points:

- It appears impossible to explain the discontinuity among individual, by supposing that each member has appeared suddenly due to mutations.
- The mutation theory was unable to explain the presence of flightless birds on oceanic islands.
- It could not explain the existence of discontinuity in distribution among individuals.
- Many mutations, described by de Vries in *Oenothera lamarckiana*, are now known to be due to certain numerical and structural changes in the chromosomes. For instance, gigas mutant of *O. lamarckiana* was later found to be due to polyploidy.
- Mutation theory alone could not explain evolution. It, however, provided raw material for other forces to act upon it and bring about evolutionary changes.
- It is difficult to believe that mutations have provided sufficient opportunity for all specialized adaptations that exist in nature.

Check Your Progress

1. Define mutation.
2. What did de Vries do?
3. List the characteristics of the theory of mutation.

14.3 POPULATION GENETICS

All the individuals of a species constitute a population. The genetical studies for the inheritance of phenotypic traits in a given population is called population genetics. The population genetics is a quantitative science. To calculate the results of the mode of inheritance of genes in a given population various statistical and

mathematical models are employed in it. Certain fundamental aspects of population genetics are the following:

Mendelian Population

A population of a particular species includes many inbreeding groups. The inbreeding groups may form a community within defined geographical boundaries and are called Mendelian population'. A Mendelian population, thus, is a group of sexually reproducing organisms with a relatively close degree of genetic relationship (such as species, subspecies breed, variety, strain, etc.) residing within defined geographical boundaries where interbreeding occurs.

Gene Pool and Gene Frequency

To get a F_2 3:1 phenotypic ratio of a monohybrid cross, we began with two homozygous parental strains, such as AA and aa: that is, we introduced the alleles A and a in equal frequency (A frequency is the ratio of the actual number of a individuals falling in a single class to the total number of individuals. But in a Mendelian population, frequencies of alleles may vary considerably. For example in Menedelian population old man the gene for polydactyly is dominant, yet the polydactylous phenotype is fairly infrequent among infants. This indicates that frequency of the dominant allele in population is lower than that of its recessive allele and that both alleles do not exist in population in the 1:1 ratio like the individuals of monohybrid cross.

Further, if all the gametes produced by a Mendelian population are considered as a hypothetical mixture of genetic units from which the next generation will arise, we have the concept of a gene or gamete pool. The percentages of gametes in the gene pool for a pair of alleles (A and a) depend upon the genotypic frequencies of the parental generation whose gametes form the pool. Thus, if a population is of dominant genotype AA, then the frequency of dominant alleles in the gene pool will be relatively high and the percentage of gametes bearing the recessive (a) allele will be correspondingly low.

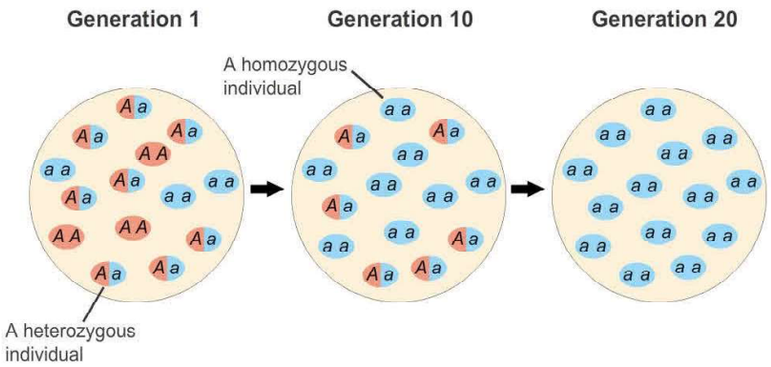

Fig. 14.2 *Showing Concept of Gene Pool*

14.3.1 Hardy-Weinberg Law

The formula $(p+q)^2=p^2+q^2+2pq$ is expressing the genotypic expectations of progeny in terms of gametic or allelic frequencies of the parental gene pool and is originally formulated by a British mathematician Hardy and a German physician Weinberg (1908) independently Both forwarded the idea called Hardy-Weinberg law equilibrium after their names, that 'both gene frequencies and genotype frequencies will remain constant from generation to generation in an infinitely large interbreeding population in which mating is at random and no selection, migration or mutation occur'. Should population initially be in disequilibrium, one generation of random mating is sufficient to bring it into genetic equilibrium and thereafter the population will remain in equilibrium (unchanged in gametic and zygotic frequencies) as long as Hardy-Weinberg condition persists.

Hardy-Weinberg law depends upon the following kinds of genetic equilibriums for its full attainment:

- The population is infinitely large and mate at random.
- No selection is operative.
- The population is closed, i.e., no immigration or emigration occurs.
- No mutation is operative in alleles.
- Meiosis is normal so that chance is the only factor operative in gametogenesis.

Genetic Drift

Genetic drift (also known as allelic drift or the Sewall Wright effect) is the change in the frequency of an existing gene variant allele) in a population due to random sampling of organisms. The alleles in the offspring are a sample of those in the parents, and chance has a role in determining whether a given individual survives and reproduces. A population's allele frequency is the fraction of the copies of one gene that share a particular form. Genetic drift may cause gene variants to disappear completely and thereby reduce genetic variation (Refer Figure 14.3). It can also cause initially rare alleles to become much more frequent and even fixed. Genetic different have following characteristics:

- Genetic drift is a mechanism of evolution in which allele frequencies of a population change over generations due to chance (sampling error).
- Genetic drift occurs in all populations of non-infinite size, but its effects are strongest in small populations.
- Genetic drift may result in the loss of some alleles (including beneficial ones) and the fixation, or rise to 100% frequency, of other alleles.
- Genetic drift can have major effects when a population is sharply reduced in size by a natural disaster (bottleneck effect) or when a small group splits off from the main population to found a colony (founder effect).

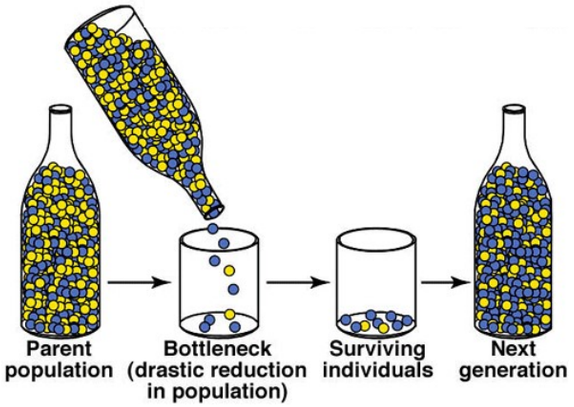

Fig. 14.3 *Concept of Genetic Drift: Bottleneck Effect*

Check Your Progress

4. What does the term population genetic mean?
5. What is Mendelian population?
6. Define genetic drift.

14.4 EVOLUTION OF PRIMATES AND HUMAN

A primate (from Latin *primat*, from *primus*: 'prime, first rank') is a mammalian animal constituting the taxonomic order Primates. Primates arose 55–85 million years ago from small terrestrial mammals (Primatomorha), who adapted to living in the trees of tropical forests: many primate characteristics represent adaptations to life in this challenging environment, including large brains, visual acuity, color vision, altered shoulder girdle, and dexterous hands. The order Primates has two suborders:

- Suborder Prosimii (Tree Shrews, Lemurs, Tarsiers)
- Suborder Anthropoidea (Monkeys, apes, Man)

Among the prosimians the tree shrews are the most primitive, resembling both insectivores and primates in structure. Indeed they were formerly classed as members of order Insectivora. The immediate descendants of these tree shrews were lemurs and tarsiers, both represented by numerous forms in early periods of the Coenozoic era. Lemurs are small animals resembling monkeys in many ways possess flattened nails instead of claws but one digit of each hand has a specialized claw). Tarsiers are represented today by only one form, the spectacled tarsier, found in some island of the East Iindies. Some of the Eocene tarsiers resembled monkeys in on and skull characteristics and hence they may well have been ancestral to higher members of the order. It is clear that early coenozoic prosimians were the ancestors of higher primates (Refer Figure 14.4).

NOTES

Suborder Antropoidea is divided into two groups:

- New World Monkeys
- Old World Monkeys Apes and Men

Man and apes lack an external tail. Most monkeys of Old World have tails which in some cases is more or less shortened, while the New World monkeys have prehensile tails.

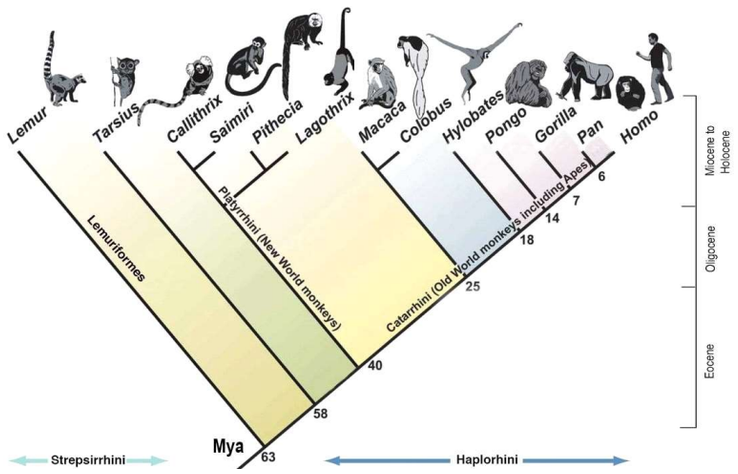

Fig. 14.4 *Osteology and Fossil Record Based Phylogenetic Tree Showing Evolution from Lemure to Modern Man*

Evolution of Monkey and Apes

The evolution of Old World monkeys, apes and men probably occurred during Eocene period of Coenozoic era. The Eocene prosimians (lemurs and tarsiers) gave rise Oligocene ancestors of Old World primates, i.e., *Parapithecus*. New World and Old World forms may have originated from different groups of Eocene prosimians. *Parapithecus* was a small creature and it is known from a single lower jaw, less than two inches in length found in Egypt. *Parapithecus* could have been the ancestors, both of Old World monkeys and of apes. The Old World monkeys begin to diverge from the rest during Oligocene. Among the apes, gibbon line separates from the rest apes in Oligocene. *Parapithecus* is a probable ancestor of the gibbon. Besides it, the two other fossils *Propliopithecus* from the Oligocene and *Pliopithecus* from the Miocene indicate the course of gibbon evolution. Modern gibbons found in south eastern Asia and the East Indies are slender bodied and long-armed. A varied assemblage of apes (subfamily Dryopithecinae) lived in various parts of Africa, Europe and India during Miocene and on into Pliocene times. They are believed to be the ancestors of the modern orangutan, chimpanzee and gorilla. The chimpanzee, and gorilla are so similar that they thought to have diverged from a common ancestor recently in early Pleistocene or late Pliocene (Refer Figure 14.5).

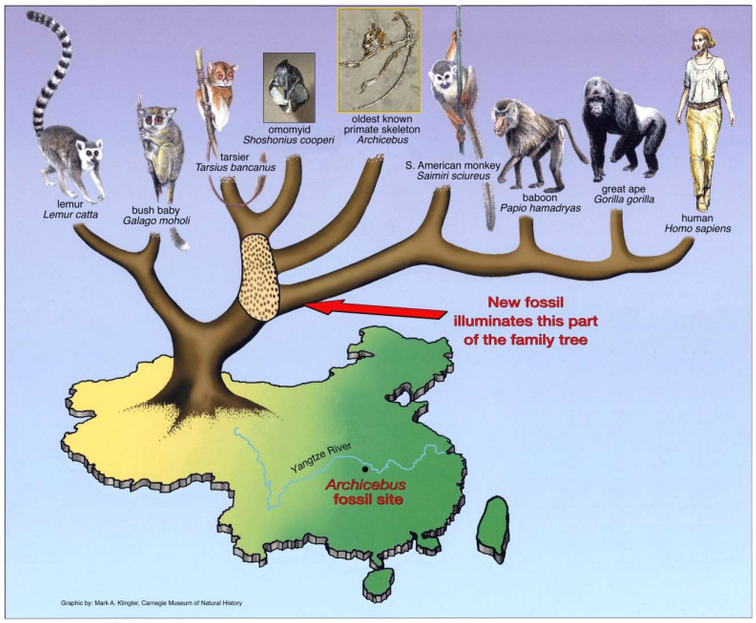

Fig. 14.5 *Family Tree illuminates Evolution of Monkey and Apes*

Evolution of Man

The *Parapithecus*, a monkey-ape was ancestral to man indicated by fossil records and characters. It possessed very primitive characters of primates and hence it represents a stage in evolution in which old world monkey, apes, and ancestors of man were not clearly separated. Man shared common ancestry with monkeys and apes and hence it was ancestral to all three. A few investigators have thought and maintained that man is not related to monkeys and apes because man has followed a separate evolutionary line springing directly from tarsiers and lemurs.

Among living primates the chimpanzee and gorilla are man's closest relatives, as judged by many similarities of structure of both skeleton and soft parts, including type of placenta and the results of serological tests. But there are also differences and the most important difference of the manner in which apes travel through the trees. Modern apes are strongly specialized for brachiation (swinging through the tree tops by their arms). Though the massive gorillas do not spend much time on the trees, but their anatomy clearly indicated that their immediate ancestors were brachiators. Among the specialization for brachiation are elongation of the forelimbs, reduction of the thumb so that the other fingers of the hand function as a sort of hook on the branches of trees, and shortening of the hindlimbs. Man is not a branchiator. Relative to trunk height both modern apes and man have long arms, however, man differs from mode apes in having longer legs than arms. In this respect, man resembles old world monkeys than that to the modern apes. Authorities differ about whether *Proconsul* itself should be regarded as ancestral to man. *Proconsul* was not a specialized brachiator (Refer Figure 14.6).

NOTES

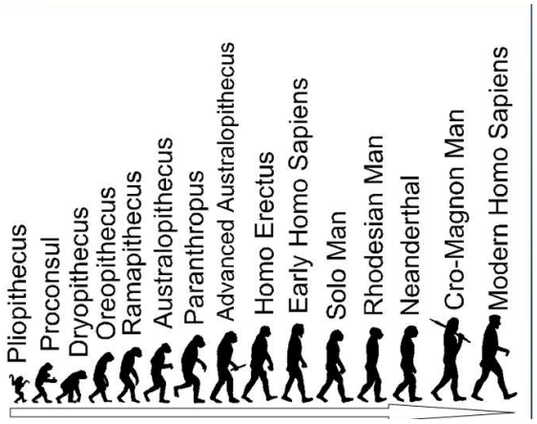

Fig. 14.6 *Schematic Diagram of Evolution of Modern Man*

Oreopithecus, a fossil primate lived in late Miocene or early Pliocene times, some thirteen million years ago. Many fossils of this have been found in a lignite mine in Tuscany Italy. *Oreopithecus* resembled man, and differed from apes and monkeys in so many characters like dentition, small face and pelvic girdle as well as pattern of walking similar to man. The earliest primate, which truly man-like creature and different from the ape, is *Ramapithecus*. Its fossils were found in India in 1930 and scientists believe that these creatures existed in Asia and Africa about 8 to 14 million years ago. They could grind their food with side to side movement of jaw bones like man and walked on floor upright like man. On the basis of majority of closeness and recent finds of primitive fossil apes indicate more similarities between men and man-like anthropoid apes which led to general theory of common ancestry. In evolution, the next primate that followed *Ramapithacus* was *Australopithecus*. It is supposed to be the most immediate ancestor of man among the primates. From Pliocene of South Africa, at Taungs was found a perfect skull, which has been called by Dart *Australopithecus african*us. It is similar to primitive apes in certain features and to man in many others like: terrestrial in habit, dominant special sense, opposable thumbs and big toes, enlarged brain and brain case, and dentition. Ultimately these evolved as man in late Pleistocene through a series of evolution (*Paranthropus*, Advanced *Australopithecus*, *Homo habilis*, *H. erectus*, early *H. sapiens*, solo man, Rhodesian man, *H. neanderthalesis*, Cro-Magnon and modern man) (Refer Figure 14.7).

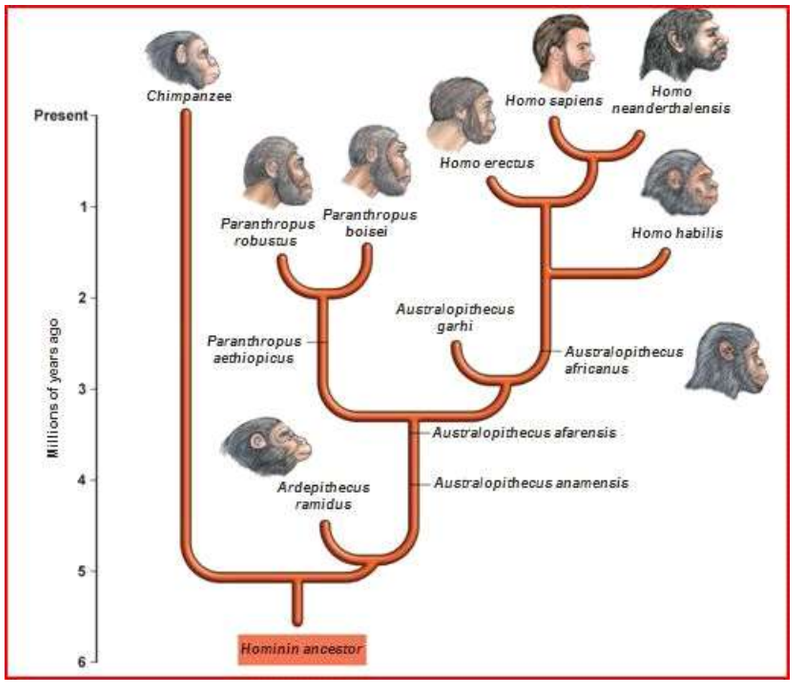

Fig. 14.7 *Cladogram Showing Evolution of Modern Man from Hominid Ancestor*

Place of Human Origin

Evidences show that the Central Asia is the place of origin of man because it was a central place for migration to other places. Europe is a place for the divergent evolution of several human species. The oldest known human remains are found in Asia, China and Java. It was the seat of oldest civilization. The Central Asia is the place of almost all of our domestic animal. The climatic conditions in Asia in Miocene or Early Pliocene were such, due to which the pre-human ancestors became compelled to descend from the trees and it was essential for the further human development Cause of Descent of Pre-human Ancestors from Trees. The pre-human ancestors descended from the trees either in the Miocene or in Early Pliocene. The descent of pre-human ancestors was probably due to geological disturbance. Therefore, they became terrestrial and started adapting themselves to the new conditions (Refer Figure 14.8).

Evolutionary Changes in Man

There were following changes noticed based on different evolutionary evidences:

- Gradual assumption of more erect posture. Forearms became liberated from locomotor function and gradually become shorter in size (shorter arms). Perfection in thumb opposability and opposable great toe became parallel to other toes.

- Development of chin prominence, loss of hairs over the body, increase in skull capacity. Increase in size and complexity of brain, especially frontal lobes.
- Reduction of muzzle (projection of head including nostrils and mouth) and size of teeth, canine became relatively shorter in size, loss of jaw power.
- Diminution in strength of zygomatic or temporal arch, and development of articulate speech.
- Due to dwindling of forests, man could not easily obtain his food, he had to search for living and so became hunter.
- Due to harsh weather during long winter he needed the clothes and hence became free from climatic restrictions and then dispersal took place from one habitat to another.

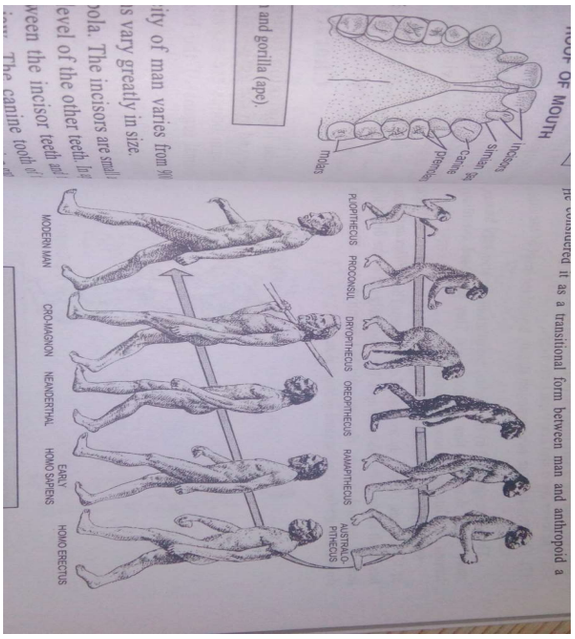

Fig. 14.8 *Showing Evolutionary Changes from Ancient Man to Modern Man*

Characteristics of Human

During the course of evolution, the man has adapted various advanced characters than monkeys and apes are summarized below:

Characteristics of Man

There are following characteristics of man responsible to separate him from monkeys and apes:

- Bipedal locomotion, upright posture, shorter arms and and perfection of thumb opposability.

- The skull is balanced on the upper end of vertebral column instead of projecting anteriorly from it. Ileum bones are expanded to form a sort of basin supporting the internal organs of the body cavity. Ilia are elongated and lack this upportive function in apes.
- Nose has a prominent ridge and well-developed elongated tip. Median furrow in his upper lip, lips are outrolled so that mucous membrane is visible as a continuous red line.
- Presence of a chin (the lower margin of the lower jaw extends forward as a chin), high forehead with projecting eyebrow ridges and body is relatively hairless.
- Brain size large size with cranial capacity 900 to 2300c.c.
- Dental arch is a smoothly rounded parabola. The incisors are small and the canines project slightly beyond the level of the other teeth. In apes there is a simian gap or diastema between the incisor teeth and the canine tooth in each side of the upper jaw. The canine tooth of the lower jaw fits into this space when the mouth is closed. Simian gap is lacking in *Homo sapiens* and his predecessors (for example, *Proconsul*) tooth row in man is short as compared to that of apes and some of the earlier hominids. The longer tooth row causes the face to produce into a short of muzzle (Refer Figure 14.9).
- The man has very long period of post natal growth.

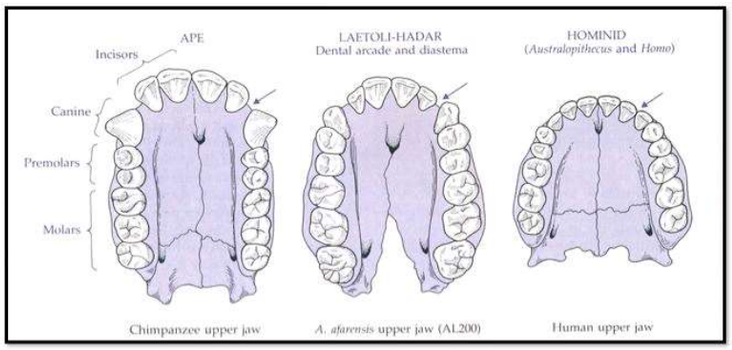

Fig. 14.9 *Comparative Account of Upper Dental Arches of Apes, Man and Modern Man*

Characteristics of Modern Man (*Homo sapiens*)

Homo sapiens first begins to appear in fossil record about 3,00,000 years ago i.e., at about the time of Java and Peking man, man of definite modern characters evolved in Europe. A Fragmentary skull, found at Swanscombe in Kent (England), is supposed to belong to *Homo sapiens*, having the brain capacity 1350cc. Another important finding of *Homo sapiens* was made in France in 1947. Africa has also produced an early *Homo sapiens*, which was as old as Swanscombe man.

Hence, *Homo sapiens* include all the existing men and also some extinct men. The modern man appeared in the second interglacial stage and was contemporary rather than a descendant of Neanderthal man. *Homo sapiens* have a number of distinctive characters in common, although in varying degree (Refer Figure 14.10). These are:

- The entirely erect posture, with four reversed curves in the spine which act as a shock-absorbing device to protect the nicely poised skull.
- The limbs are straight, but the segmental proportions vary racially and individually.
- The skull also varies in size and relative proportions, such as length to breadth.
- The forehead is generally steep and the continuous eyebrow-ridge is absent.
- The final distinctive feature is the protruding chin prominence, the result of the reduction of the dental arch.

Fig. 14.10 *Modern Man*

Homo habilis a nut-cracker man or able man was discovered Dr. Louis Leakey from Olduvai in the form of the remains of five fossil hominids and a lower jaw. He lived about 17,50,000 years ago. Leakey believed that this man had lived with

Zinjanthropus and *Pithecanthropus* and made tools. He was 110 to 140cm in height, walked erect and its cranial capacity was near about 700cc. His teeth were more human like. Recently P. Boriskovsky quoted that *Homo habilis* of east Africa was more ancient (2,600,000 years old) than *Pithecanthropus* of Java.

Characteristics of Future Man

The man is little subjected to the laws which govern adaptations of animals in their environment. Man has adapted to speed, flight, to fossorial and aquatic. But his adaptation is artificial. In animals adaptation is natural and the stamp of the environment is deeply impressed upon organism. The man's physical evolution has virtually ceased or reached an end point, but if any change is being effected, it is largely retrogressive. Such retrogressive changes are:

- Reduction of hair and teeth, and of hand skill.
- Senses of sight, smell and hearing will become more efficient in response to the selective force of a high speed society.
- Bones of skull will generally become thinner because of the reduced stress from the jaw muscles.
- Face will become generally more refined and beautiful because of reduced physical stress and because of the action of sexual selection.
- Forehead will be larger and the eyes set more deeply. Nose will be more prominent and narrow. Mouth will be smaller, but the chin will be more prominent.
- Body will be slender. Breasts of the female will be smaller.

Man is hardly subjected to Malthus's law owing largely to the one of the young which makes the expectation of life of the new-born relatively very high, his migratory ability and above all his intelligence save him from the application of law. A single new discovery such as electricity may increase his food supply and other life necessities several times. His future evolution will be mental and spiritual rather than physical. Mankind is burdened with the care of the unfit, and their genes, which would be eliminated by natural selection in any other species as are found. Hence biological disaster is inevitable. We live in a high speed, mechanized society. Hence people who are physically and mentally adapted to live in such a society should in the long run, have a selective advantage.

Check Your Progress

7. What is a primate?
8. What does order Primates have?
9. In how many groups suborder Antropoidea divided?

14.5 MOLECULAR PHYLOGENETICS

Molecular phylogenetics is the branch of phylogeny that analyzes genetic, hereditary molecular differences, predominately in DNA sequences, to gain information on an organism's evolutionary relationships. From these analyses, it is possible to determine the processes by which diversity among species has been achieved.

The result of a molecular phylogenetic analysis is expressed in a phylogenetic tree. Molecular phylogenetics is one aspect of molecular systematics, a broader term that also includes the use of molecular data in taxonomy and biogeography. Molecular phylogenetics and molecular evolution correlate. Molecular evolution is the process of selective changes (mutations) at a molecular level (genes, proteins, etc.) throughout various branches in the tree of life (evolution). Molecular phylogenetics makes inferences of the evolutionary relationships that arise due to molecular evolution and results in the construction of a phylogenetic tree. The figure displayed on the right depicts the phylogenetic tree of life as one of the first detailed trees, according to information known in the 1870s by Haeckel.

The early attempts at molecular systematics were also termed as chemotaxonomy and made use of proteins, enzymes, carbohydrates, and other molecules that were separated and characterized using techniques such as chromatography. These have been replaced in recent times largely by DNA Sequencing, which produces the exact sequences of nucleotides or bases in either DNA or RNA segments extracted using different techniques. In general, these are considered superior for evolutionary studies, since the actions of evolution are ultimately reflected in the genetic sequences. At present, it is still a long and expensive process to sequence the entire DNA of an organism (its genome). However, it is quite feasible to determine the sequence of a defined area of a particular chromosome (conserved gene). Typical molecular systematic analyses require the sequencing of around 1000 base pairs. At any location within such a sequence, the bases found in a given position may vary between organisms. The particular sequence found in a given organism is referred to as its haplotypes. In principle, since there are four base types, with 1000 base pairs, we could have 4^{1000} distinct haplotypes. However, for organisms within a particular species or in a group of related species, it has been found empirically that only a minority of sites shows any variation at all, and most of the variations that are found are correlated, so that the number of distinct haplotypes that are found is relatively small (Refer Figure 14.11).

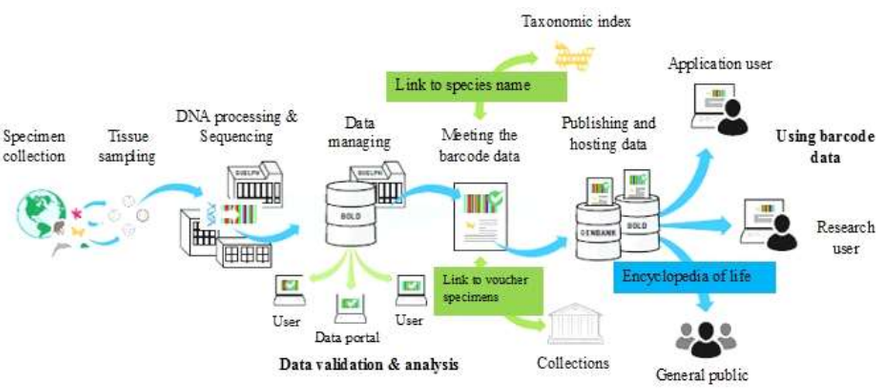

Fig. 4.11 *Outline for Tools and Techniques Employed in Molecular Phylogeny*

An older and superseded approach was to determine the divergences between the genotypes of individuals by DNA-DNA hybridization. The advantage claimed for using hybridization rather than gene sequencing was that it was based on the entire genotype, rather than on particular sections of DNA. Modern sequence comparison techniques overcome this objection by the use of multiple sequences. Once the divergences between all pairs of samples have been determined, the resulting matrix of differences is submitted to some form of statistical cluster analysis, and the resulting dendrogram is examined in order to see whether the samples cluster in the way that would be expected from current ideas about the taxonomy of the group. Any group of haplotypes that are all more similar to one another than any of them is to any other haplotype may be said to constitute a clade (Refer Figure 14.12).

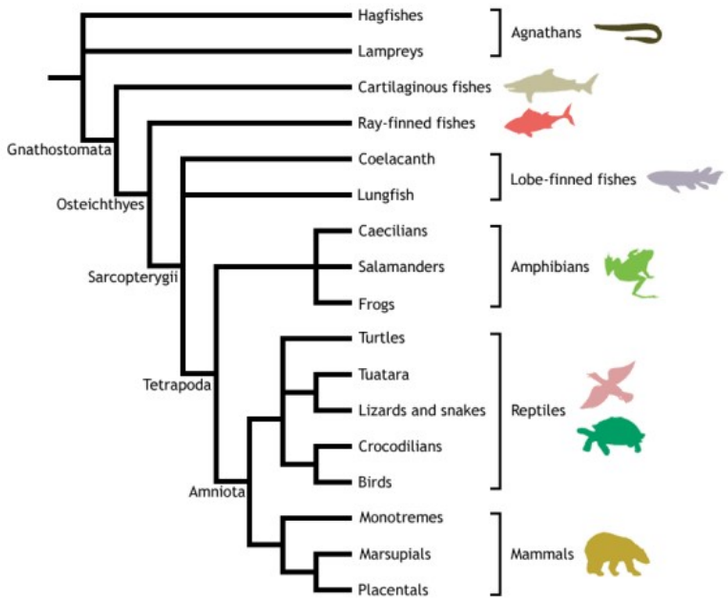

Fig. 14.12 *Cladogram of Different Vertebrates*

Applications and Techniques

Every living organism contains Deoxyribonucleic Acid (DNA), ribonucleic acid RNA, and proteins. In general, closely related organisms have a high degree of similarity in the molecular structure of these substances, while the molecules of organisms distantly related often show a pattern of dissimilarity. Conserved sequences, such as mitochondrial DNA, are expected to accumulate mutations over time, and assuming a constant rate of mutation, provide a molecular clock for dating divergence. Molecular phylogeny uses such data to build a 'relationship tree' that shows the probable evolution of various organisms. With the invention of Sanger sequencing, it became possible to isolate and identify these molecular structures. The most common approach is the comparison of homologous

sequences for genes using sequence alignment techniques to identify similarity. Another application of molecular phylogeny is in DNA barcoding, wherein the species of an individual organism is identified using small sections of mitochondrial DNA or chloroplast DNA. Another application of the techniques that make this possible can be seen in the very limited field of human genetics, such as the ever-more-popular use of genetic testing to determine a child's paternity, as well as the emergence of a new branch of criminal forensics focused on evidence known as genetic finger printing.

Molecular Phylogenetic Analysis

There are several methods available for performing a molecular phylogenetic analysis. One method, including a comprehensive step-by-step protocol on constructing a phylogenetic tree, including DNA/Amino Acid contiguous sequence assembly, multiple sequence alignment, model-test (testing best-fitting substitution models), and phylogeny reconstruction using Maximum Likelihood and Bayesian Inference (Refer Figure 14.13).

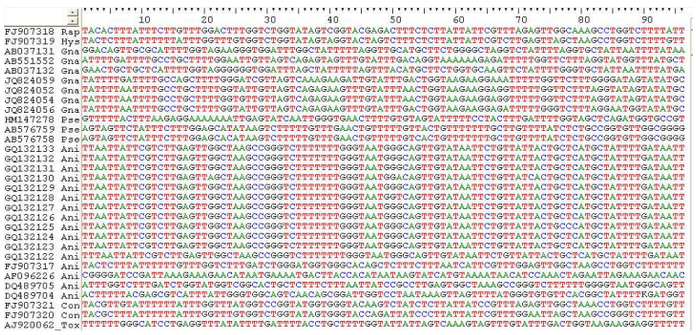

Fig. 14.13 *Multiple Sequence Alignment (MSA) by Software BioEdit*

A phylogenetic analysis typically consists of five major steps. The first stage comprises sequence acquisition. The following step consists of performing a multiple sequence alignment, which is the fundamental basis of constructing a phylogenetic tree. The third stage includes different models of DNA and amino acid substitution. Several models of substitution exist. A few examples include Hamming distance, the Jukes and Cantor one-parameter model, and the Kimura two-parameter model. The fourth stage consists of various methods of tree building, including distance-based and character based methods. Common tree-building methods include unweighted pair group method using arithmetic mean (UPGMA) and Neighbor joining, which are distances based methods, Maximum parsimony, which is a character-based method, and Maximum likelihood estimation and Bayesian, which are character-based/model based methods. UPGMA is a simple method; however, it is less accurate than the neighbor-joining approach. Finally, the last step comprises evaluating the trees. This assessment of accuracy is composed of consistency, efficiency, and robustness (Refer Figure 14.14.

Fig. 14.14 *Five Stages of Molecular Phylogenetic Analysis*

MEGA (molecular evolutionary genetics analysis) is analysis software that is user-friendly and free to download and use. This software is capable of analyzing both distance-based and character-based tree methodologies. MEGA also contains several options one may choose to utilize, such as heuristic approaches and bootstrapping. Bootstrapping is an approach that is commonly used to measure the robustness of topology in a phylogenetic tree, which demonstrates the percentage each clade is supported after numerous replicates. In general, a value greater than 70% is considered significant (Refer Figure 14.15).

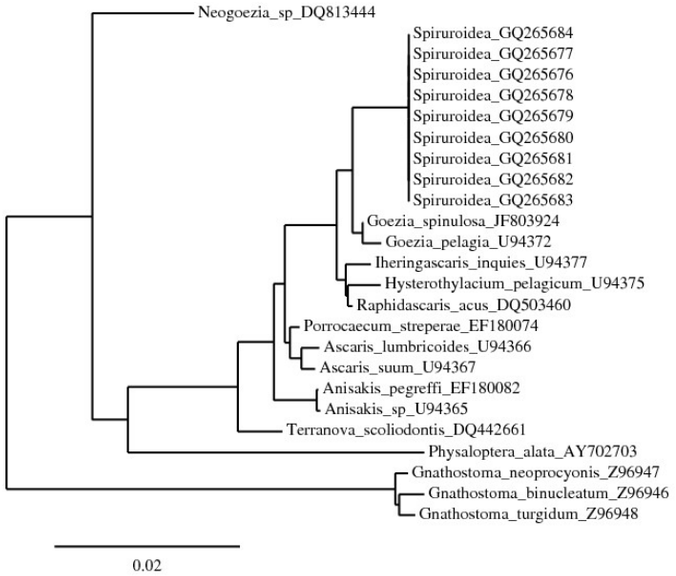

Fig. 14.15 *Neighbor Joining Tree Computed by Kimura's Two Parameter (K2P) Model by MEGA*

Limitations of Molecular Phylogeny

Molecular systematics is an essentially cladistic approach: it assumes that classification must correspond to phylogenetic descent, and that all valid taxa must be monophyletic. This is a limitation when attempting to determine the optimal tree(s), which often involves bisecting and reconnecting portions of the phylogenetic tree(s). The recent discovery of extensive horizontal gene transfer among organisms provides a significant complication to molecular systematics, indicating that different genes within the same organism can have different phylogenies. In addition, molecular phylogenies are sensitive to the assumptions and models that go into making them. They face issues such as long-branch attraction, saturation, and taxon sampling problems. This means that strikingly different results can be obtained by applying different models to the same dataset. Moreover, as previously

mentioned, UPGMA is a simple approach in which the tree is always rooted. The algorithm assumes a constant molecular clock for sequences in the tree. This is associated with being a limitation in that if unequal substitution rates exist, the result may be an incorrect tree.

Check Your Progress

10. Define molecular phylogenetics.
11. What is molecular evolution?
12. How can a molecular phylogenetic analysis conducted?
13. What does a phylogenetic analysis consists of?

14.6 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Mutation is a hereditary change in the genetic make-up of an organism other than that, which may be caused by the simple recombination of genes. These include the changes in the gene structure or composition (gene mutations or point mutations) and the changes in the chromosomes either in number or structure (chromosomal mutations).
2. de Vries recognized the following seven distinct species of *Oeonothera*:
   - *O. gigas* (giant and stout plants with large flowers and deep green leaves)
   - *O. rubrinervis* (fruit red veined, leaves pale green and stem slender and britle)
   - *O. oblonga* (dwarf and weak plants with oblong leaves)
   - *O. albida* (weak plants with whitish-pale leaves)
   - *O. laevifolia* (leaves narrow and smooth and pale flowers)
   - *O. brevistylis* (round leaves, flowers with very short style and flattened stigma)
   - *O. vanilla* (very short or dwarf variety; leaves sessile)
3. The characteristics of the theory of mutation are as given below:
   - Mutations appear from time to time among the organisms of a naturally breeding species or populations. The organisms with mutations are called mutants. These mutants are clearly distinct from their parents.
   - Mutations are heritable and form new species. They do not disappear by crossing.
   - Mutations are sudden and large and are different from Darwin's fluctuating variations, which are small and directional.

- Mutations may appear in any direction, and are subjected to natural selection.
- Unsuitable mutants are destroyed by natural selection.
- Mutations appear full-fledged and hence there is no question of incipient stages in the development of an organ.
- Mutation occurs in all possible direction and may be advantageous or disadvantageous.

4. The population genetics is a quantitative science. To calculate the results of the mode of inheritance of genes in a given population various statistical and mathematical models are employed in it.
5. A population of a particular species includes many inbreeding groups. The inbreeding groups may form a community within defined geographical boundaries and are called Mendelian population.
6. Genetic drift (also known as allelic drift or the Sewall Wright effect) is the change in the frequency of an existing gene variant allele) in a population due to random sampling of organisms.
7. A primate (from Latin *primat*, from *primus*: 'prime, first rank') is a mammalian animal constituting the taxonomic order Primates.
8. The order Primates has two suborders:
   - Suborder Prosimii (tree shrews, lemurs, tarsiers)
   - Suborder Anthropoidea (monkeys, apes, man)
9. Suborder Antropoidea is divided into two groups:
   - New World monkeys
   - Old World monkeys apes and men
10. Molecular phylogenetics is the branch of phylogeny that analyzes genetic, hereditary molecular differences, predominately in DNA sequences, to gain information on an organism's evolutionary relationships.
11. Molecular evolution is the process of selective changes (mutations) at a molecular level (genes, proteins, etc.) throughout various branches in the tree of life (evolution).
12. There are several methods available for performing a molecular phylogenetic analysis. One method, including a comprehensive step-by-step protocol on constructing a phylogenetic tree, including DNA/Amino Acid contiguous sequence assembly, multiple sequence alignment, model-test (testing best-fitting substitution models), and phylogeny reconstruction using maximum likelihood and Bayesian Inference.
13. A phylogenetic analysis typically consists of five major steps. The first stage comprises sequence acquisition.

NOTES

14.7 SUMMARY

- Dutch Botanist Hugo de Vries was born at Haarlem, studied at Heidelberg and also Wurgberg. He published book entitled '*Die Mutation Theorie*' and proposed the mutation theory in order to explain the mechanism of evolution based on observations in evening primrose, *Oenothera lamarckiana.*
- Mutations appear from time to time among the organisms of a naturally breeding species or populations. The organisms with mutations are called mutants. These mutants are clearly distinct from their parents.
- C. Darwin recognized two kinds of variations as material for natural selection. These variations were mainly ever present fluctuating (continuous), which played dominant role in species formation.
- Mutations are regarded as the ultimate source of new and different genetic material appearing in a population, or the genetic mechanism producing the change is known as a mutation.
- Mutations are the basis of discontinuous variation in populations. Mutations arise spontaneously and are not directed by the environment.
- Linnaeus was a systematicist not an evolutionist, his objective being to place all known organisms into a logical classification which he believed would reveal the great plan used by the Creator- the *Systema Naturae*.
- A population of a particular species includes many inbreeding groups. The inbreeding groups may form a community within defined geographical boundaries and are called Mendelian population'.
- A Mendelian population, thus, is a group of sexually reproducing organisms with a relatively close degree of genetic relationship (such as species, subspecies breed, variety, strain, etc.) residing within defined geographical boundaries where interbreeding occurs.
- The formula $(p+q)^2=p^2+q^2+2pq$ is expressing the genotypic expectations of progeny in terms of gametic or allelic frequencies of the parental gene pool and is originally formulated by a British mathematician Hardy and a German physician Weinberg (1908) independently.
- The Old World monkeys begin to diverge from the rest during Oligocene. Among the apes, gibbon line separates from the rest apes in Oligocene. *Parapithecus* is a probable ancestor of the gibbon.
- Modern apes are strongly specialized for brachiation (swinging through the tree tops by their arms). Though the massive gorillas do not spend much time on the trees, but their anatomy clearly indicated that their immediate ancestors were brachiators.

- The pre-human ancestors descended from the trees either in the Miocene or in Early Pliocene. The descent of pre-human ancestors was probably due to geological disturbance.
- Molecular evolution is the process of selective changes (mutations) at a molecular level (genes, proteins, etc.) throughout various branches in the tree of life (evolution).
- Molecular phylogenetics makes inferences of the evolutionary relationships that arise due to molecular evolution and results in the construction of a phylogenetic tree.
- Molecular systematics is an essentially cladistic approach: it assumes that classification must correspond to phylogenetic descent, and that all valid taxa must be monophyletic.

14.8 KEY WORDS

- **Mutation:** Mutation is a hereditary change in the genetic make-up of an organism other than that, which may be caused by the simple recombination of genes.
- **Genetic drift:** Genetic drift (also known as allelic drift or the Sewall Wright effect) is the change in the frequency of an existing gene variant allele) in a population due to random sampling of organisms.
- **Alleles:** An allele is a variant form of a given gene. Sometimes, the presence of different alleles of the same gene can result in different observable phenotypic traits, such as different pigmentation.
- **Point mutations:** Changes in the structure of genes are point mutations.

14.9 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short Answer Questions

1. Give some of the characteristics of mutations.
2. Brief a note on advantages of mutation theory.
3. Write a short note on gene pool and gene frequency.
4. Give a general account on Hardy-Weinberg law.
5. How did evolution of monkey and apes occurred?
6. What are the evolutionary changes in man that are seen?
7. Write a short note on molecular phylogenetic analysis.

Long Answer Question

1. Write an essay on mutation theory.
2. What are mutations? Discuss the importance of mutations.
3. Explain Hugo De Vries theory of organic evolution and discuss its merits and demerits.
4. Discuss the origin and evolution of *Homo sapiens*.
5. Give an account of the fossil history of man and the trends in the human evolution with particular reference to recent fossil records.
6. Write an essay on molecular phylogenetic analysis.

14.10 FURTHER READINGS

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